Studies on the biology of selected commercial pelagic teleosts, grey mullets, <italic>Chelon labrosus</italic> (Risso) and <italic>Liza ramada</italic> (Risso) and herring, <italic>Clupea harengus</italic> L.

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Studies on the biology of selected commercial pelagic teleosts, grey mullets, *Chelon labrosus* (Risso) and *Liza ramada* (Risso) and herring, *Clupea harengus* L.

by

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To my parents

ABSTRACT

Aspects of the biology of grey mullets, thick-lipped, *Chelon labrosus* Risso and thin-lipped, *Liza ramada* Risso, populations of the coastal waters of the Bristol Channel were studied. A qualitative and quantitative examination of stomach contents of *C. labrosus* revealed a feeding transition from copepods in juveniles to a diet consisting of benthic crustacea, microalgae and detritus together with sediments in the larger fishes. The change in diet occurred at a 6-8 cm S.L.

Investigations of population dynamics indicated that the females of *C. labrosus* and *L. ramada* were heavier than the males. The overall male to female ratio in *C. labrosus* and *L. ramada* was found to be 1:1.1 and 1:1.2 respectively. Seasonal variation in the sex ratio was discussed. The Gonadosomatic Index indicated April to May to be the spawning period for *C. labrosus* and December to January for *L. ramada*. Age and growth of *C. labrosus* was determined and the von Bertalanffy Growth equation was fitted which indicates that this fish is a slow growing and long-lived species.

The ultrastructural changes during oocyte development of *C. labrosus* were described, and seven stages of oogenesis identified. Vitellogenesis commenced with the appearance of lipid yolk droplets in stage 2 (20-110 μm diameter) oocytes. Lipid yolk and cortical alveoli were endogenous in origin, formed by golgi complexes and endoplasmic reticulum. Protein yolk granules first appeared in stage 4 (200-250 μm diameter) oocytes and were formed from both endogenous and exogenous sources.

Coastal waters receive run-off from the land and effluent discharge and will therefore be contaminated with pollutants including heavy metal ions. Eggs and larvae of *Clupea harengus* L. may be subjected to the

pollutants as this species spawns in coastal waters. Artificially fertilized eggs of *C. harengus* exposed to copper concentrations of 0.01, 0.03 and 0.05 ppm, showed a reduction in the total egg volume and perivitelline space volume. In the newly hatched larvae jaw, branchial and vertebral column deformities were observed. Ultrastructural changes in the brain, epidermal and muscle cells of the larvae, induced by copper were mainly swelling of the endoplasmic reticulum, degeneration of mitochondria, vacuolation in the epidermal cells and reduction in myofibrils of muscle cells.

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Chapter 1

General Introduction

GENERAL INTRODUCTION

Thick-lipped grey mullet, *Chelon labrosus* (Risso), and thin-lipped grey mullet, *Liza ramada* (Risso), belong to the family Mugilidae which are Actinopterygian teleosts. Their close relatives are the Barracudas (Sphyraenidae), and Silversides (Atherinidae) from which they differ in their teeth not being implanted in sockets and in having only a rudimentary lateral line or none at all. Excluding fossils, fourteen genera and 281 nominate species of mugilids have been recorded, of which Thomson(1964) recognised 70 as valid though others remain indeterminate because of inadequate descriptions. The majority of mugilids occur in the Indo-Pacific region where there are 10 genera and 49 species, compared with 2 genera and 6 species in the northeastern Atlantic, 3 genera and 9 species in the southeastern Atlantic, 2 genera and 7 species in the western Atlantic and 3 genera and 5 species in the eastern Pacific (Thomson, 1966). *C. labrosus* and *L. ramada* are common along the Atlantic coasts from the Azores and Madeira northward to the British Isles, Southern Norway, the Faroes and Southern Iceland. They also occur in the whole of the Mediterranean but are rare in the Black Sea. Both species are abundant along the coastal area of the Bristol Channel (Claridge and Potter, 1985), the area of the present study. Anderson (1982) and Wheeler (1969) produced a key for the identification of the British grey mullets. *C. labrosus* differs from *L. ramada* by having a thick upper lip (its greatest depth more than half the eye diameter) with a papillated lower margin and has six pyloric caecae, while *L. ramada* has a thin upper lip (its greatest depth less than half the eye diameter) without papillae and has seven pyloric caecae. They have been named differently by different workers. *C. labrosus* is also named *Mugil*

labrosus (Risso), *M. chelo* (Risso), *Crenimugil labrosus* (Risso), *M. corrugatus* (Lowe) and *M. septentrionalis* (Gunther), while *L. ramada* is also called *M. ramada* (Risso), *M. capito* (Cuvier), *M. dubahra* (Valenciennes), *M. petherici* (Gunther) and *Myxus macroccensis* (Mohr)(Hureau and Monod, 1979).

The grey mullets constitute an important, and probably the most widely distributed, commercial fishery in the world's coastal waters. They make a significant contribution to the subsistence protein requirements of the people of the Pacific Basin, southeastern Asia, India, the Mediterranean and eastern European countries and in many parts of central and south America. Mullet and mullet products contribute a valuable fishery in the economies of many European countries, the southern United States of America, Japan and Australia (Nash and Shehadeh, 1980).

The biology of the grey mullet has been reviewed in detail by Thomson (1966). Hickling (1970) studied food, reproduction and population dynamics of grey mullet in the English Channel. Kennedy and Fitzmaurice (1969) studied the age and growth of *C. labrosus* in Irish waters and Claridge and Potter (1985) reported the size and distribution of *C. labrosus* and *L. ramada* in the Bristol Channel. *C. labrosus* and *L. ramada* are euryhaline fish, able to penetrate into waters with a wide range of salinities. Both species live in the neritic region of the pelagic zone, lying between the littoral and benthic zone where the well lit waters with plentiful nutrients from river and upwelling areas result in an enormous production of algae, sea grass and plankton. *C. labrosus* and *L. ramada* spend most of the time in estuaries but also migrate to deeper seawater in winter for spawning (Hickling, 1970).

Estuaries are habitats which overlap the littoral and sublittoral zones, are productive regions which often serve a valuable function as

'nursery grounds' for young or feeding or breeding grounds for adult fish. Pitchard (1967) defined an estuary as a semi-enclosed coastal body of water which has a free connection with the open sea and within which seawater is measurably diluted with fresh water derived from land drainage. Estuaries are the most dynamic of environments in that conditions within them such as temperature, salinity and turbidity vary more widely, rapidly and frequently than in other aquatic environments (Arther, 1972).

During the hydrological cycle, water falling on the land as rain, enters the estuaries by river inflow or by run-off from the land during which this water may gain substances leached from the soil or products of anthropogenic origin. Most of the substances transported by river water are contaminating agents and are generally adsorbed to particulate matter (Huiskes and Rozema, 1988). Lauff (1967) and Willey (1976) called estuaries sinks for particulate matter, some of which may be pollutants. Fish and other organisms living part or all their lives in estuaries may thus be exposed to these substances.

Pollutants have been defined as any substance in the environment which results in measurable harmful effects on living organisms (Hynes, 1960; Martin, 1976; Goulden, 1978; Clark, 1986). Some heavy metals and other trace elements, radioactive elements and chlorinated hydrocarbons are accumulated by organisms, such that considerable concentrations are built up in the tissues of the organisms from the concentrations in seawater. An accumulation of all those elements or organic compounds have no positive metabolic function, but they may manifest harmful effects (Gerlach, 1981).

Pollutants may enter the bodies of marine animals in a variety of ways. Uptake is either from seawater, from food or from sediments or by physical contact with suspended particles. Especially in filter-feeding

animals in which large quantities of sediments pass through their guts. These sediments are sometimes mixed with waste industrial by-products (Webb, 1973). Animals which ingest polluted sediments may be affected. Most of the grey mullet species are detritus and sediment feeders and may thus accumulate pollutants in their bodies. For example, in the Derwent Estuary, Tasmania, *Mugil cephalus* L. carried a zinc concentration of 38.9 ppm which was higher than the concentration in fish from the same location with pelagic feeding habits (Eustace, 1974). Establier (1975) found similar levels of mercury in sediments and in the bodies of *M. cephalus* in Cadiz Bay. Sidhu *et al.* (1972) showed that Australian common grey mullet, *M. cephalus* with a relatively high body fat content is likely to take up petroleum hydrocarbons more readily than other species of fish living in the same environment. Hydrocarbons in the flesh of these grey mullet originated from the uptake of polluted sediments of waters close to oil refineries (Alverez-Lajonchere, 1974). Caesium was accumulated in grey mullet feeding on algae containing this pollutant (Isaac, 1975). Since accumulation of substances may occur in commercially important fish species, it is important to know what effects these substances may have on the fish.

Pollutants may affect reproduction of organisms in many different ways. The development of embryos may result in deformed or malfunctioning larvae which do not survive hatching. Reproduction may be influenced by behavioural changes of the adults during the mating season. Their behaviour, the production of eggs, the secretion of egg membranes and production of egg nutrients may all be affected by changes of hormone function and enzyme activity. Brungs (1969) and Mount and Stephan (1969) noticed the complete inhibition of reproduction and retarded maturation of *Pimephales promelus* Rafinesque, when the fish was exposed to copper and zinc

solutions. Reproductive failure, retarded sexual development and spawning was prevented in *Salvelinus fontinals* Mitchill, and *P. promelus* Rafinesque exposed to hard fresh water containing copper (Mount, 1968; McKim and Benoit, 1971). A high mortality of embryos, premature hatching and smaller larvae resulted when eggs of *C. harengus* were exposed to cadmium (Rosenthal and Sperling, 1973). Similar effects were also observed when eggs of plaice, *Pleuronectes platessa* L. and herring, *C. harengus* and trout, *S. fontinalis* were exposed to copper (McKim and Benoit, 1971; Blaxter, 1977). Eggs and larvae of *C. harengus* were affected by acid water ($FeSO_4$ and H_2SO_4), resulting in a reduced percentage of successful fertilization and egg survival, reduced diameter of fertilized eggs, retarded embryonic growth rate, shortened duration of incubation, decreased percentage of successful hatching and structural abnormalities of hatched larvae (Kinne and Rosenthal, 1967).

Unlike many contaminants, some heavy metals are normal constituents of the marine and estuarine environments and at least trace amounts are usually present in organisms living in these environments. The term 'heavy metal' includes all metals and metalloids with an atomic number greater than 23 and densities greater than 5 gcm^{-3}. Some exist as a part of the earth's crust (Duffus, 1980; Mason, 1981). Some of these in trace amounts, are essential to the normal metabolism of organisms; others are considered not to be required (non-essential) and may be toxic even in small amounts (Bryan, 1971; Thorp and Lake, 1974). The non-essential group of metals includes Ag, Hg, Cd, Al and Pb. In animals these metals have the undesirable characteristics of being not only deadly, entirely without any apparent useful compensating function in biological systems, but they regularly occur as contaminants. Their presence in living tissue is usually a reflection of

their presence in the environment, and is not that of a cellular requirement (Tucker, 1972).

Fourteen metals are believed to be essential for animal life, they are Fe, I, Cu, Mn, Zn, Co, Mo, Se, Cr, Sn, F, Si, Ni and V. Underwood (1975) defined an element as considered essential if its deficiency consistently results in an impairment of functions from optimal to sub-optimal levels. The most obvious and readily quantifiable functions of essential metals is somatic or body growth, while their deficiency results in growth impairment particularly in young organisms.

Essential metals serve their biological function satisfactorily only when they are present within specific limits and in particular forms, so that either the deficiency or the over-abundance of an essential heavy metal can lead to disorder or to toxic effects (Hubschman, 1967; Bryan, 1971; Tucker, 1972).

The concentration of a heavy metal which is toxic to an aquatic organism is dependent both on the particular metal and on the organism. Bryan (1971) stated that mercury, silver and copper are the most toxic metals, followed by cadmium, zinc, lead, chromium, nickel and cobalt. This order of toxicity, though, is different for different species (Bryan, 1971). The uptake of heavy metals to produce an accumulation or internal concentration greater than in the external environment appears to be widespread in aquatic organisms (Witton and Say, 1975). When a toxic substance enters a marine organism it may be metabolized into a different toxic substance (Halstead, 1972). Poisoning effects of heavy metals on fish have been reported by several workers (Lloyd, 1962; McKim and Benoit, 1971; Alderdice *et al.*, 1979; Somasundaram *et al.*, 1984a,b,c; Klauda and Palmer, 1987; Thomson *et al.*, 1988).

As for most heavy metals, copper only occurs in minute amounts in natural waters, but additional copper enters estuaries and coastal waters as a result of mine drainage, mineral ore separation or smelting operations (Abdullah and Royle, 1972; Chubb *et al.*, 1979; Forstner and Wittman, 1981). Chester and Stoner (1974) found an average copper concentration of 0.8 ppb in the open ocean and 0.9 ppb nearer the shore. In British waters Preston *et al.* (1974) reported a range of 1.1-1.6 ppb and Portmann (1972a) found a range of 1-20 ppb with a possibility of 1000 ppb near sea-bed copper deposits.

Copper is one of the essential metals in organisms for many physiological processes including oxygen transport and electron transport systems. It forms a part of many enzymes (e.g., tryrosinase) and is an activator for others (e.g., melate dehydrogenase). It is an important element for the synthesis of haemoglobin, formation of bone and maintenance of myelin within the nervous system (Ghiretti, 1962; Adrien Albert, 1965; Takeshimurai *et al.*, 1981).

In teleosts, high levels of copper are known to be lethal, may cause functional derangement and anatomical changes (Gardner and La Roche, 1973; Gardner, 1975; Finlayson and Verrue, 1980; Steele, 1985; Reid and McDonald, 1988), however, low levels of copper are essential for metabolism. Copper may enter the fish from solution or from ingested material and concentrates in the tissues of the fish (Bryan, 1971). In fish, the liver is the major storage organ for accumulated copper. Later it distributes to other internal tissues such as white muscles, the kidney, spleen, gut and brain (Lauren and McDonald, 1987b). Anderson *et al.* (1973) found 3.4 - 7.2 ppb dry weight of copper in *C. harengus* in Oslofjord. Topping (1973) reported .55 - 1.17 ppb wet weight copper in fish from Scottish waters.

Fish exposed to solutions of copper sulphate suffer a dilation of the kidney tissue, renal necrosis around the tubules, destruction of hematopoitic tissue, increased liver fat, a copper residue in their gills, muscles and liver (Klink, 1975). Copper is also known to inhibit specific enzymes in fish (Jackim *et al.*, 1970; Lorz and Mcpherson, 1976), damage gills (Baker, 1969; Gupta and Rajbauski, 1981), liver (Gardner and La Roche, 1973; Wong *et al.*, 1977; Singh, 1985), skin (Gardner and La Roche, 1973; Wong *et al.*, 1977), kidney (Cardeilhac *et al.*, 1979), stomach and gut (Singh, 1985) and cause increased permeability of mitochondria (Zaba and Harris, 1978). Carpenter (1927, 1930) reported that the minnow, ***Leuctsus phoxinus***, when immersed in copper, lead, zinc or other heavy metal solutions died as a result of the formation of an insoluble and impermeable layer covering the gill membrane and body surface which hindered respiration of the fish. Sublethal concentrations of copper altered the ultrastructure of gills and liver in fish by damaging the cells (Baker, 1969; Segner, 1987).

In several studies copper was a toxic element for fish eggs and larvae. Blaxter (1977) reported a decrease in the survival of embryos of herring, *C. harengus*, at 0.03 ppm copper concentration and all the hatched larvae in this concentration were deformed. He suggested that copper is most harmful in the very early egg stages. Shazili and Pascoe (1986) exposed eggs and alevins of rainbow trout, *Salmo gairdneri* Richardson, in copper, cadmium or zinc. They observed an increase in sensitivity of embryos to copper throughout the embryonic development, but in the case of cadmium and zinc alevins were more sensitive than eggs immediately prior to hatching. In some studies the sensitivity of embryos and larvae was not uniform throughout development, being dependent on the stage of development

and the metal to which it is exposed (Skidmore, 1965; Eaton *et al.*, 1978; Rombough and Garside, 1982; Shazili and Pascoe, 1986).

Since these effects of pollutants were observed in fish, particularly species which occur in inshore and estuarine waters (Baker, 1969; Alderdice and Forrester, 1971; Somasundaram *et al.*, 1984; Elliott *et al.*, 1988; Bahgat *et al.*, 1989), a study of grey mullet, *C. labrosus* and *L. ramada*, was considered of importance. A study of their feeding habits, population structure and reproductive biology would be necessary to form a basis for work on the effects of pollutants. In most species of fish the young stages are most affected by pollutants (Hazel and Meith, 1970; Rosenthal and Sperling, 1974; Giles and Klaverkamp, 1982; Rombough and Garside, 1982; Shazili and Pascoe, 1986) but, because mullet spawn offshore (Hickling, 1970; Oren, 1981), their eggs and larvae would not be subjected to inshore and estuarine pollution. With the facilities available during the study it was not possible to obtain eggs and larvae of mullets so it was decided to use eggs and larvae of the herring, *C. harengus*, a pelagic teleost which spawns inshore and in estuaries. Its' eggs are demersal and the larvae grow and develop in shallow water where pollution occurs. It was assumed that eggs and larvae of this species may give some indication of how eggs and larvae of mullet may react to the pollutants used in the study. In previous work eggs and larvae of herring have been tested with other substances (Kinne and Rosenthal, 1967; Blaxter, 1977; Somasundaram *et al.*, 1984a,b, 1985; Somasundaram, 1985; Klauda and Palmer, 1987; Bahgat *et al.*, 1989).

Chapter 2

Food of thick-lipped grey mullet, *C. labrosus* in natural waters

INTRODUCTION

Grey mullet, (family Mugilidae), are euryhaline fish distributed throughout offshore and inshore waters in many parts of the world. Many aspects of their biology including food and feeding habits, growth, migration, reproduction and early development have been reported (Thomson, 1966; Hickling, 1970; Pillay, 1972; Kuo *et al.*, 1974; Cato and McCullough, 1976; Blaber and Whitefield, 1977; Marais and Erasmus, 1977). There are a considerable number of dietary studies of grey mullets from estuaries and coastal waters of different regions (Jacot, 1920; Hiatt, 1944; Pillay, 1953; Thomson, 1954, 1966; Luther, 1962; Suzuki, 1965; Odum, 1970; Hickling, 1970; Romer and McLachlan, 1986; and Wijeyaratne and Costa, 1987). These studies have shown that the diets of different species of grey mullet vary considerably. Zismann *et al.* (1974) stated that young stages of grey mullet increase in size offshore and when the juveniles approach the beaches, estuaries and lagoons, their feeding pattern changes and they begin to feed on a variety of phytoplankton and zooplankton. Juvenile grey mullet feed on surface planktonic material (Zismann *et al.*, 1974; Ferrari and Chieregato, 1981; Collins, 1981; Kraul, 1983; Romer and McLachlan, 1986), whilst the diet changes again later and the adult grey mullet feed on benthic organisms (Hiatt, 1944; Pillay, 1953; Odum, 1968a; Blaber, 1976). The diet of the most widely occurring common grey mullet, ***Mugil cephalus*** L. has been extensively studied (Thomson, 1966; Odum, 1970). The larval stage of ***M. cephalus*** is carnivorous, as it starts feeding on surface zooplankton on the fifth day of hatching (Suzuki, 1965; Zismann *et al.*, 1974; Kuo *et al.*, 1973). Other grey mullet species, ***Liza richardsoni*** and ***L. subviridis*** are also carnivorous and consume zooplankton during the larval stage (Chan and

Chua, 1979; Romer and McLachlan, 1986), although postlarval *M. tade*, *M. cephalus*, *L. malinoptera*, *M. parsia* and *M. speigheri* have been observed to feed on phytoplankton (Pillay, 1953; Sarojini, 1954; De Silva and Wijeyaratne, 1977; Ching, 1977). The change in diet of juvenile *M. cephalus* was noticed when the fish grow through a length of 23-33 mm standard length (SL). Primarily they are surface zooplankton feeders then mixed food feeders (zooplankton and benthic detritus) and finally the juveniles over 55 mm SL are benthic feeders feeding on plant material (diatoms, green algae and blue green algae)(Suzuki, 1965; Zismann *et al.*, 1974). Similar diet changes also occur in juvenile *Liza aurata*, *Liza ramada* and *Liza saliens* (Albertini-Berhaut, 1974). Wijeyaratne and Costa (1987) observed that juvenile *Liza tade*, of 5 cm total length feed on similar food as the adults, feeding heavily on benthic detritus and zooplankton.

Adult mullet are benthic feeders but have also shown differences in their diet in different localities and among different species. Thomson (1966) described three categories of food items of adult *M. cephalus*, (1) microalgae, including epiphytic and benthic forms; (2) decaying plant detritus; and (3) inorganic sediment particles. Hiatt (1944) stated that the diet of Hawaiian mullet consists mainly of diatoms and blue-green algae. Luther (1962) reported that the main food of adult *M. cephalus* in Pullamadam lagoon in India is decayed organic matter, benthic algae and benthic foraminifera. *M. cephalus* from St. Lucia Lake (South Africa) appears to prefer feeding on the gastropods and foraminifera rather than on plant material (Blaber, 1976). *M. cephalus* and *Liza dumerili* from the Swartkops estuary feed on a mixed diet of plant and animal material (Masson and Marais, 1975). Farrugio (1976) recorded differences in feeding habits of grey mullet in Tunisian lakes. *C. labrosus* and *L. ramada* were mainly

carnivorous feeding on benthic crustacea whereas *M. cephalus* had a herbivorous diet feeding on benthic diatoms and foraminifera. According to Kreft and Michaelis (1976), the diet of *C. labrosus* is of the mixed types, herbivorous (diatoms and cyanophycea) and carnivorous (Nematoda and harpacticoid copepods). The carnivorous feeding habits of adult *M. cephalus*, which feed heavily on polychaetes have been described by Bishop and Miglarese (1978) from South Carolina.

Three species of grey mullet have been recorded in British waters, namely, the thick-lipped grey mullet, *Chelon labrosus* Risso, the thin-lipped grey mullet, *Liza ramada* Risso, and the golden mullet, *Liza aurata* Risso. *C. labrosus* and *L. ramada* are abundant in the Bristol Channel (Claridge and Potter, 1985). The present study describes qualitative and quantitative analyses of the diet of juvenile and adult, thick-lipped grey mullet, *C. labrosus* occurring in the Bristol Channel.

Materials and Methods

(i) *Sampling Procedure*

The immature fish were sampled from Oldbury Power Station in the inner Severn Estuary and from Hinckley Point in the inner Bristol Channel. The fish obtained from Oldbury were fixed on site in 5% buffered seawater formalin during November and December 1987. Fresh fish collected from Hinckley Point at monthly intervals were put in ice or fixed in 5% buffered seawater formalin and transported to the laboratory as soon as possible.

The adult fish <35 cm total length, caught by gillnet in Rhossili Bay, Gower (Fig. 1), were purchased at regular monthly intervals from a local fisherman. The fish were kept in ice and transported to the laboratory for morphometric study before opening the viscera to examine the gut contents.

(ii) *Analysis of Stomach Contents*

The method applied to analyse the stomach content of the juvenile fish was used by Ferrari and Chieregato (1981). Because of the large size range of juveniles, specimens were divided into six size groups and analysed to detect changes in diet, if any. The six groups were:-

(i) 3.0 to 3.6 cm SL (Standard length); (ii) 3.7-4.4 cm SL; (iii) 4.5-5.1 cm SL; (iv) 5.2-6.0 cm SL; (v) 6.1-8.0 cm SL; (vi) 8.1 SL and over. The stomach contents from each group were pooled for analysis. The plant and animal organisms present were identified to the species level whenever possible. The occurrence of each food item in the samples of stomach content was categorised on a scale None (N), Occasional (O), Common (C) and Abundant (A).

The stomach contents of adult fish were analysed by the method of Pillay (1953) and Hiatt (1944) used for food analyses of *Mugil tade* and *M. cephalus* respectively. Most samples of food were taken from the cardiac portion of the stomach. Due to the decayed organic matter and the algal matrix present, it was not possible to apply a numerical method of analysis or to count the fragments of algal matter. By volumetric analysis, however, it was found possible to assess with a fair amount of accuracy the composition of food items. According to Hynes (1950), until dietetic values of food species are known, volume forms a satisfactory basis for assessment, when the food of the animal investigated consists of vegetable matter, wholly or in part.

The stomach contents were thoroughly washed into petri dishes containing adequate quantities of water for dilution and examined under the microscope. Each food component was identified to the species level whenever possible and listed in the following major categories: (i) Animal

matter (amphipods, copepods and other animals); (ii) Detritus (decomposed plant and animal material); (iii) Inorganic matter (sand, silt and mud); (iv) Diatoms; and (v) Algae (chlorophyta and cyanophyta).

Pillay (1953) preferred the volumetric analysis of the food of *M. tade* rather than the counting method due to the nature of the food. The stomach contents were analysed by two methods, (i) Percentage composition by volume (the volume of food items of a given type that occurred in all specimens examined, expressed as a percentage of the total volume of all food items); (ii) Percentage frequency of occurrence (the percentage of non-empty stomachs containing one or more examples of a particular food item). Attempts were made to separate the chief constituents of the stomach, so that a correct volumetric assay could be made. The volume of each food item was calculated by mixing in a known volume of water in a volumetric cylinder and described as the percentage of the whole food eaten. In order to quantify sand, silt and mud, it was separated by filtering the food on fine meshed cloth by adding more water as needed. Then the turbid water was poured into a volumetric flask and left for several hours to set the mud at the bottom of the flask.

Feeding condition was determined by the degree of distension of the cardiac portion of the stomach and the amount of food it contained. The fullness of the stomach was classified as (i) Gorged (the walls of stomach distended with food); (ii) Full (stomach cavity full but walls not distended); (iii) ½-full; (iv) ¼-full; (v) Traces (containing a little food); and (vi) Empty (Pillay, 1953). The time of the collection for each sample was noted and feeding intensity was determined by the fullness of the stomach at different times.

Observations:

(i) Food of Juveniles of C. labrosus.

C. labrosus is an offshore spawner, no larvae or post-larvae have been recorded in British coastal waters (Kennedy and Fitzmaurice, 1969; Claridge and Potter, 1985). The smallest juveniles captured during the present study were of 3.0 cm standard length. Table 1 shows the food items in the stomach of all size groups of juveniles from 3.0 cm SL to 8.,0 cm SL and over. The juveniles of 3.0 to 4.4 cm SL mostly utilized copepods and a few diatoms. Harpacticoid copepods, calanoid copepods, copepod nauplii and cirripede larvae were taken. Diatoms such as *Melosira, Chaetoceros, Skeletonema, Nitzschia, Pleurosigma, Bacillaria* and *Licmophora* were present in the food. The stomachs of juveniles 4.5 to 5.1 cm SL contained copepods and indicated an increased intake of diatoms. In some stomachs of this group, terrestrial insects (Diptera adults with developed wings) were present but due to their occasional presence were not included in Table 1. The size groups 5.2 to 6.0 cm SL and 6.2 to 8.0 cm SL fed on mixed type of food consisting of copepods, diatoms and chlorophyta and cyanophyta and detritus. Some traces of sand and silt were also present. The size group 8.2 cm SL and over fed mostly on benthic copepods and other crustaceans and detritus mixed with sand and silt. The nature of their food shows benthic feeding.

(ii) Food of adult C. labrosus.

The qualitative and quantitative analyses of the stomach contents is presented in Table 2 and Figure 4. Stomach contents showed that animal matter, decayed matter mixed with sand, silt and mud formed the main food of the fish. Algae appeared to be taken in along with the food items associated with them when the fish feed on the bottom. Fish eggs and larvae were also seen occasionally in a very small quantity and were not a regular

item of the food. The food components identified in the diet (Table 2) are as follows:

(a) Animal Matter:-

Animal matter formed one of the major constituents of the diet. This category comprises amphipods, mysids, cumaceans, copepods and molluscs. Amphipods included *Phtisica, Acaudata, Dulichia, Scina, Arigissatypica, Hyprta* and *Metopa*. Among the mysids, *Sirrila, Gastrossaccus, Neomysisitteger* and *Mesopodopsis* were present. *Eurydice* sp., *Diatylis* sp. and *Cuma* sp. were mostly taken among the cumaceans. Adult benthic copepods such as *Oithona, Centropages, Temora, Candacia, Calanus* sp. were utilized. The larvae of Gastropods, *Mytilus edulis* and *Spiratella* sp. were common in the food. Sometimes fish had full stomachs of *Mytilus edulis* larvae. Polychaetes occurred occasionally, such as bristle worms approx. 10 cm long. The maximum uptake of living organisms were observed during May to August.

(b) Detritus:-

Detritus consisted of a decayed slimy mass of unrecognizable plant material mixed with the appendages of crustaceans and mud. After washing and removing the crustacean appendages it appeared as a white jelly-like substance probably the plant mucus. The maximum quantity of detritus was taken in November to April.

(c) Algae:-

Diatoms, chlorophyta and cyanophyta were identified in the diet. The most common diatoms present in the stomach were *Nitzschia, Thalassiothrix, Pleurosigma, Chaetoceros, Asterionella, Bacillaria, Navicula, Striatella* and *Bacteriastum*. Among the chlorophyta and cyanophyta, *Oscillatoria, Skujaella, Nostoc, Spirogyra, Lyngbya, Spirulina, Aphanizomenon, Coelosphaerium, Chlorella, Traubaria, Tetraspora, Protococcus* and *Mongeotia*

were taken in the diet. Dinoflagellates of the *Ceratium* sp. were also present in some of the stomachs, but was not a regular item of the diet. The maximum quantity of algae occurred in the diet between July and September.

(d) Inorganic Matter:-

Inorganic matter consisted of sand, silt and mud. Very fine grains of sand and dark-coloured mud mixed with detritus formed a regular item in the stomach. Mud was present in considerable quantities occurring in the range 5 to 20% of the total food volume (Table 2).

The monthly percentage by volume of the three major food components living organisms, detritus and inorganic matter is shown in Table 3 and Figure 3. Living organisms both animal and plant material were a regular major food item throughout the study. The period of maximum consumption of living organisms was May to August, ranging from 44.6% to 52% of the total food volume. However, from September to April they made up only 30% of the total food volume. During the study fish with gorged stomachs contained mostly animal matter, indicating that the fish preferred to feed on living organisms particularly animal matter, rather than plants. Detritus amounted to 20 to 31.4% during the months of May and August. The quantity of detritus in the diet increased up to 37 to 45% during September to April, maybe due to unavailability of living organisms in sufficient amounts. Inorganic matter consisting of mud, sand and silt made up 23.6% of the total food volume and remained about the same throughout the period of study.

The monthly feeding condition is indicated in Table 4. In the sample (45 fish) captured in August 1988 in the early morning at 5 a.m., none of the specimens had a gorged stomach, only 13.3% were half full and 46.6% were empty. In the sample (36 fish) captured in June 1987 at 7 a.m., on the

other hand, the percentages were 19.47 gorged, 25% full and 27.7% empty. This indicates that the fish start feeding after 5 a.m. The sample (40 fish) captured at 9 a.m. in April and June 1988 had a gorged or full stomach but no stomach was absolutely empty, which suggests that this is the peak feeding time. The fish had fed actively until 3 p.m. and feeding decreased in the sample (26 fish) captured at 8 p.m. in May 1988. The results indicate that the fish start feeding in the morning and carry on throughout the day, but feeding decreases at night.

The percentage of occurrence of food items in the stomach is shown in Table 5. Detritus, animal matter and mud, sand and silt formed regular constituents of the diet and occurred in the stomachs of most of the specimens examined. Algae were found in only a small number of stomachs and were not an important food item. Detritus was present in 97.5% of all stomachs, animal matter in 86.7% and sand and silt in 96.4%. Algae was present in 28.3% of all stomachs examined.

DISCUSSION

In the present study the feeding habits of juvenile *C. labrosus* change as they grow. Juveniles 3.0 to 4.4 cm SL had a planktonic diet, feeding mostly on harpacticoid copepods, copepod nauplii, calanoid copepods and diatoms. A change in diet took place when the juveniles reached 5.2–8.0 cm SL, then feeding on planktonic organisms including both zooplankton and phytoplankton mixed with traces of sand and silt and detritus. The juveniles larger than 8 cm SL were bottom feeders feeding on amphipods, mysids and other benthic crustaceans, benthic algae and detritus together with sand and silt. Hickling (1970) reported that juvenile *C. labrosus* arrived in British coastal waters at a length of 2.0 to 3.0 cm SL and fed on

epiphytic diatoms and harpacticoid copepods. No detritus was present in their diet. The juvenile *C. labrosus* in the semi-enclosed fish reservoir of the Bassin d'Arcachon with lengths of 1.5-5.0 cm SL mostly feed on pelagic organisms including the calanoid copepods, various harpacticoid and cyclopoid copepods associated with algae (*Cladophosa* and *Enteromorpha*)(Lasserre *et al.*, 1975). A change in diet is also reported in the juveniles of other species of mullet. Albertini-Berhaut (1975) reported three types of diet in *M. capito*, juveniles (1.5-2.5 cm SL) feed on crustaceans, larger individuals (2.5-5.5 cm SL) feed on mixed food consisting of animal and plant while large ones (>5.5 cm SL) feed on a compound of plant material (algae, benthic diatoms) and sediments. Egusa (1950) pointed out that *M. cephalus* starts feeding on benthic amphipods and harpacticoid copepods on reaching a body length of 2.1-3.9 cm. The diets of juveniles of *M. aurata, M. capito* and *M. saltens* (measuring 1.5-6.6 cm SL) from the Gulf of Marseilles consist mainly of benthic crustaceans, harpacticoid copepods (Albertini-Berhaut, 1974). In *L. saltens*, juveniles <3 cm SL feed on zooplankton (Cirripedia nauplii, neritic Copepoda, Polychaeta larvae), small Amphipoda and adult insects; juveniles >3 cm SL feed on benthic elements (Nematoda, Polychaeta, algae) and zooplankton particularly nauplii of copepods and cirripeda, and juveniles over 5 cm SL feed on sand and silt, diatoms and filamentous algae, Nematoda and Polychaeta (Ferrari and Chieregato, 1981). The juveniles of several mullet species after entering estuaries along the eastern coast of South Africa, change their diet from zooplankton to benthic zooplankton (1.0-1.5 cm) to meiobenthos (1-2 cm) and finally (1.5-2.5 cm) to sand particles and associated microbenthos (Blaber and Whitefield, 1977). Romer and Mclachlan (1986) also noticed the change in diet of *L. richardsoni*, from zooplankton

to mixed food of animal and plant to benthic algae and beach sediments. Pillay (1953) suggested that in *M. tade*, the change in diet occurred with the development of the processes of the gill rackers and the pharyngeal teeth which enabled the fish to sieve the food matter from mud. Blaber and Whitefield (1977) concluded that the estuaries play a major role in providing suitable conditions for juvenile mullet to change their feeding habits to that of the adults which occurs soon after the fish enter estuaries. This change in diet is only possible in shallow and quiet waters of estuaries, because seawater is usually rough and it would be difficult for juvenile mullet of smaller size to consume sand and associated benthic organisms under conditions of substrate movement in the inshore waters.

In the present study adult *C. labrosus* were bottom feeders. The fish utilised animal matter, detritus, sand and silt and benthic microalgae. The proportion of algal uptake was very low compared to the other food items. During the study it was noticed that usually fish had full stomachs containing only benthic crustaceans (amphipods, mysids, cumaceans or copepods) or the larvae of *Mytilus edulis*. Amphipods buried in mud were sucked in with large amounts of mud. However, the present study suggests that *C. labrosus* consumes a mixed diet containing animal matter and detritus as the chief components. Lasserre *et al.* (1975) reported that in the diet of adult *C. labrosus*, the dominant constituents were members of the meiofauna (nematodes, harpacticoid copepods, ostracods, oligochaetes, polychaetes, ciliates and turbelarians) and macrofauna (*Nereis*, chironomid larvae, *Hydrobia*, *Abra* and *Corophium*). Green and blue-green algae and diatoms were also present in notable quantities in the diet. Kreft and Michaelis (1976) reported that the diet of *C. labrosus* generally consisted of a mixed type, herbivorous (diatoms and cyanophycea) and carnivorous

(Nematoda and harpacticoid copepods). Hickling too, stated (1970) that in the diet of *C. labrosus*, the largest food items were the amphipods (*Corophium* and *Gammarus*), harpacticoid copepods and soil nematodes. Algae (diatoms, green and blue-green algae) and detritus (organic matter) were also a part of the diet.

According to Blaber (1976), *M. cephalus* preferred feeding on gastropods rather than on plant material. Polychaetes and detritus were the most important food items of *L. tade* throughout all sizes of the fish over 5 cm total length (Wijeyaratne and Costa, 1987). Bishop and Miglarese (1978) reported that *M. cephalus* is a carnivorous fish, feeding on whole individual polychaetes of size 2 to >4 cm. *M. cephalus* in estuarine waters fed on detritus and sand and shell particles but, in fresh water, algae and sediment particles were dominant in the diet of the fish (Collins, 1981). *M. tade* heavily fed on detritus in an estuarine environment whilst algae were the major food item in brackish waters and lagoons (Pillay, 1953). Thomson (1954) stated that grey mullet, *M. cephalus, M. georgii, L. argentea, L. dussumieri, Aldrichetta forsteri* and *Myxus elongatus* ingest whatever organic food of suitable size is common in the area in which feeding takes place.

In the present study and in the previous reports (Hickling, 1970; Lasserre *et al.*, 1975; Kreft and Michaelis, 1976), it was observed that detritus, sand and silt were in considerable quantity in the stomach contents of *C. labrosus*. Baier (1935) suggested that the nourishment from detritus particles comes from the bacteria involved in decomposition rather than the detritus itself, whereas other workers suggest that sand particles in the food of grey mullet function to grind up food particles in the

pyloric portion of the stomach (Pillay, 1953; Thomson, 1966; Hickling, 1970; Blaber, 1976; Wijeyaratne and Costa, 1987).

In the present study the uptake of living organisms was reduced during winter, November through to March, and was replaced by the uptake of detritus (mostly of plant origin). Pillay (1953) reported that *M. tade* utilized maximum decayed matter during the season of heavy rainfall. Payne (1976) noticed that during the dry season the proportion of detritus declined but increased during the rainy season in the grey mullet species, *L. falcipinnis, L. dumerili , M. cephalus, M. curema* and *L. grandisquamis*. Wijeyaratne and Costa (1987) stated that the seasonal fluctuation in food components of *L. tade* was due to availability of that particular food item. However, Hiatt (1944) did not see any change in uptake of food items in *M. cephalus*.

In the present study the feeding intensity was deduced by the amount of food material present in the cardiac stomach. The results showed that *C. labrosus* fed actively during the day but activity reduced at night. Feeding activity has been variously described for other species by several workers. Collins (1981) and Silva and Wijeyaratne (1977) reported that in *M. cephalus*, peak feeding intensity occurred at midday. Odum (1970) stated that the feeding intensity in *M. cephalus* was not always the same, but increased with the high tides and decreased with low tides. Blaber (1976) observed in several grey mullet species, occurring in St. Lucia Lake, South Africa, that a peak of feeding activity occurred at *ca.* 1800 hours, with feeding continuing throughout the night.

Correlation between alimentary canal and nature of food of grey mullets

In most fish species the structure of the regions of the alimentary canal such as mouth, pharynx, oesophagus, stomach, intestine and related organs are adapted to their nature of food (reviewed by Hoar *et al.*, 1979). In grey mullets, the alimentary canal is morphologically well adapted for selection, ingestion and digestion of their preferred food items. The protrusible and suctorial mouth is adapted for sucking in food material and sediments from a muddy bottom (Pillay, 1953). The efficient pharyngeal filter formed by pharyngeal cushions, pharyngeal teeth and the gill rackers is suited to select and retain fine and minute food components such as plankton (Thomson, 1966; Ching, 1977). The numerous taste buds also help pharyngeal filter by identifying sediments with high amounts of microalgae, decaying detritus and other micro organisms (Odum, 1970; Ching, 1977). According to the nature of food Ching (1977) suggested that the presence of fine detrital and inorganic particles in the food of juveniles of *Liza malinoptera* (Valenciennes) indicates their use of the pharyngeal device, but the low percentage of inorganic particles suggests that the device is not fully operational. It was concluded that the pharyngeal device is functional when the fish attains a length of 4.4 cm when the change to 'adult feeding' becomes more apparent (i.e., more inorganic particles and less planktonic algae).

Grey mullets lack any obvious oral masticatory device but the gizzard-like stomach is well adapted for triturition function (Pillay, 1953). The gizzard-like stomach is also present in other fish species such as *Coregonus, Osphromenus* and *Chanos* and are microphagus, detritivores or herbivores (Hoar *et al.*, 1979). Morphologically the stomach of grey mullets consists of two regions, an anterior gizzard-like pyloric region or the

pylorus and a posterior conical cardiac region (Thomson, 1966). The wall of the cardiac region is thick and muscular but in the gorged and expended condition it becomes extremely thin and transparent. The pyloric part is exceedingly thick and muscular with a narrow cylindrical lumen (Pillay, 1953; Ching, 1977) (Fig. 2). Marais (1980) suggested that a large stomach and wide intestine in *Liza dumerili* and *Liza tricuspidens* could be advantageous, ingesting large amounts of sand together with relatively small quantities of very nutritious microalgae but the small stomach in *M. cephalus* and *Liza richardsoni* ingests the finest material.

At the anterior end of the pyloric stomach a pyloric valve guards the opening to the intestine and into this region the pyloric caeca open which vary in number from species to species (Thomson, 1966; Ching, 1977). Marais (1980) found the small number of two and least developed caeca in *M. cephalus* which had a longer intestine compared to *L. dumerili*, *L. tricuspidens* and *L. richardsoni* which contains six to seven caecae and a smaller intestine. In *C. labrosus* six pyloric caecae are present. the significance of the number of pyloric caeca is unknown. Kawai and Ikeda (1971) suggested that they are probably absorptive, digestive and storage in function.

The intestine of grey mullets is also adapted to the nature of food. Collin (1983) reported difference in length of intestine of *M. cephalus* of the same size collected from fresh water and estuaries. Fish with a short intestine in estuaries feed mostly on detritus, but in fresh water with a longer intestine feed on algae. In *L. malinoptera*, the length of intestine was about 3¼ times the length of the body (Ching, 1977). Odum (1970) suggested that an intestine to fish length ratio of 3.2:1 (for *M. cephalus*) is adequate to assimilate a diatom diet, while a longer intestine is needed

to extract nourishment from plant detritus and blue green algae. de Groot (1971) reviewed the morphological variations in flatfish guts in relation to diet and found that the relative length is greatest in Soleidae which ingest smaller polychaetes, molluscs and crustaceans, and least sized gut in Psettodidae and Bothidae which eat fish and larger invertebrates. Grey mullets have been observed to be omnivorous or herbivorous and probably that is the reason they have large intestines (Romer and McLachlan, 1986; Wijeyaratne and Costa, 1987). Hickling (1970) suggested that the longer gut of larger *C. labrosus* may be adapted to food which contains a higher proportion of indigestible matter. He measured the gut length as about five times the length of the body of *C. labrosus*. Ching (1977) said that the long intestine in *L. malinoptera* ensures a greater efficiency in digestion and absorption of vegetable matter, however, the relative gut length of juveniles is shorter and this probably correlates with a diet that is lower in plant fragments.

TABLE 1. FOOD ITEMS PRESENT IN THE STOMACH OF JUVENILE THICK-LIPPED GREY MULLETT, *C. LABROSUS*.

Food items	Size group (Standard length cm) 3.0-3.6	3.8-4.4	4.5-5.1	5.2-6.0	6.2-8.0	8.2 and over
Cirripede larva	C	A	O	O	O	O
Harpacticoid copepods	A	A	A	A	A	O
Copepod nauplii	A	A	A	A	A	O
Calanoid copepods	C	A	A	A	O	O
Crustacean (mysids, amphipods, cumacea)	O	O	C	C	C	A
Mollusca	N	N	N	O	O	C
Polychaetes	N	N	N	N	N	O
Algae (Diatoms)						
Pleurosigma	N	N	O	C	C	C
Bacillaria	O	O	O	C	C	C
Nitzschia	C	C	C	O	O	O
Navicola	N	N	C	C	O	O
Licmophora	O	C	C	C	O	O
Biddulphia	N	N	O	O	O	C
Skeletonema	O	C	C	C	C	C
Chaetoceros	C	C	C	C	C	C
Melosira	N	N	C	C	C	C
Green and Blue/Green Algae						
Spirogyra	N	N	O	O	O	C
Skujaella	N	N	O	O	O	O
Nostoc	O	C	C	C	C	C
Lyngbya	O	O	O	O	O	C
Oscillatoria	N	N	O	C	C	C
Chlorella	O	C	C	C	C	C
Anabaeria	N	N	C	C	C	C
Detritus	N	N	O	C	C	A
Sand and silt	N	N	O	O	O	A

Key: N = None; O = Occasional; C = Common; A = Abundant.

TABLE 2. THE QUALITATIVE AND QUANTITATIVE COMPOSITION OF THE GUT CONTENTS OF ADULT THICK-LIPPED GREY MULLET, *CRENIMUGIL LABROS*

ANALYSED DURING 1987-1988.

	June 1987		July 1987		August 1987	
	% age	Food Components	% age	Food Components	% age	Food Components
Animal matter	30.9	Amphipods: *Scina, Hyperia galba Eucopia, Phtisica, Metopa alderi* Mysids: *Sirriella, Gastrossaccus Neomysis integer, Mesopodopsis* Copepods: *Oithona, Candacia, Temora, Cyclopina longicorius.* Cumacean: *Diastylis, Pseudocuma, Eurydice gimaldi.*	29.0	Copepods: *Temora, Pseudocalanus Microcalanus, Eurytemora velox Paracalanus, Oithona, Cyclopina.* Mysids: *Neomysis integer, Mesopod-opsis, Praunus flexuosus, Petalo-phthalmus.* Amphipods: *Eucopia, Metopa, Stenothoe, Parathemisto* sp., *Hyperia* sp.	37.7	Copepods: *Candacia, Temora Pseudocalanus, Microcalanus Eurytemora, Oithona, Paracc* Amphipods: *Metopa, Stenoth Phtisica, Scina, Argissa.* Mysids: *Siriella, Gastrosac Paramysis arenosa.* Cumacear *Eurydice pulchra, Diastylis Cumella pygmaea.*
Decayed matter	30.5	Jelly-like decomposed substance.	30.0	- - -	27.5	- - -
Mollusca	9.3	*Mytilus edulis* larvae, Gastropods.	1.5	*Spiratella retroversa Ensis siliqua* Shell fragments.	1.5	*Mytilus edulis* larvae *Cerithiopsis tubercularis Spiratella.* Shell fragments
Mud	4.0	- - -	6.0	- - -	6.2	- - -
Diatoms	4.0	*Bactriastum Pleurosigma Thalassiothrix Nitzschia Chaetoceros*	14.0	*Lepto cylindrus Bacteriastrum Chaetoceros Nitzschia Thalassiothrix*	8.1	*Nitzschia Chaetoceros Thalassiothrix Bacteriastrum Navicula, Pleurosigma*
Algae (Chlorophyta & cynophyta)	2.1	*Mongeotia Oscillatoria Skujaella Nostoc*	7.5	*Mongeotia Skujaella Nostoc, Oscillatoria Coelospharium*	3.5	*Oscillatoria Skujaella Nostoc* Dinoflagellates (*Ceratium*)
Sand & Silt	16.0	- - -	12.0	- - -	15.5	- - -
Eggs	1.0	Copepod and fish eggs.				

	Nov. 1987		Mar. 1988		April 1988	
	% age	Food Components	% age	Food Components	% age	Food Components
Animal matter	29.5	Mysids: *Siriella* sp., *Paramysis arenosas, Siriella leptomysis* Amphipods: (Hyperiids), *Hyperia galba, Hyperoche kroyeri, Scina borealis, Phtisica acaudata, Apherusa, Argissa typica.* Copepods: *Centropages* sp., *Temora longicornis, Candacia, Calanus* sp.	22.0	Copepods: *Centropages* sp., *Paracalanus, Parvus, Temora longicorius, Cyclopoid.* Mysids: *Gastrosaccus sanctus, Siriella armata.* Amphipods: *Hyperia galba, Parathemisto* sp., *Phtisica acaudata, Metopa alderi.*	30.0	Copepods: *Paracalanus, Microcalanus, Euchaeta, Metridia* sp. *Amphipods: Parathemisto* sp., *Phtisica acaudata, Paratylus* sp., *Scina, Metopa alderi.* Mysi *Siriella* sp., *Praunus* sp., *Mysiodosis* sp., *Petalophth* Cumacean: *Eurydice pulchra*
Decayed matter	44.0	- - -	45.0	- - -	40.5	- - -
Mud	10.0	- - -	8.0	- - -	5.0	- - -
Diatoms	2.0	*Nitzchia Striatella Chaetoceros Bacillaria*	2.0	*Chaetoceros Nitzschia Bacillaria Pleurosigma Navicula*	4.2	*Thalassiothrix Asterionella notata Chaetoceros Nitzschia Bacillaria*
Mollusca	0.0	- - -	4.4	(Gastropods) *Spiratella* sp. Lamellibranch larvae *Cerithiopsis tubercularis* shells.	3.0	Shell fragments. Gastropod larvae.
Algae (Chlorophyta & cynophyta)	2.0	*Gloeotrichia Aphanizomenon Nostoc Oscillatoria Skujaella*	2.6	*Treubaria Anabaena Nostoc Oscillatoria* Dinoflagellates	1.8	*Treubaria Nostoc Oscillatoria Chlorella Spirogyra*
Sand & Silt	12.5	- - -	17.0	- - -	15.5	- - -

	May 1988		Jun. 1988		July 1988	
	% age	Food Components	% age	Food Components	% age	Food Components
Animal matter	39.6	Amphipods: *Argissa typica, Stenothoe marina, Apherusa clevei Scina, Eurythenes, Phtisica acaudata Dulicia porrecta,* Calanoid and cyclopoid copepods, Cumacean. Polychaetes.	35.5	Polychaetes (Bristleworms): *Phyllodoce muculata, Lumbriconeries latereilli, Callizona setosa, Nereis diversicolor*. Mysids: *Gastrosaccus sanctus, Leptomysis* sp., *Schistomysis* sp., *Sirtella* sp.	38.0	Copepods: calanoid and cyclopoid. Polychaetes an appendages of mysids and amphipods. A large quanti *Eurydice* sp. of cumacean.
Decayed matter	31.4	White jelly-like material	24.5	- - -	27.0	- - -
Mollusca	5.0	Broken pieces of shells. Gastropod larvae. *Mytilus edulis* larvae.	2.5	Gastropod larvae and broken shells.	4.0	*Mytilus edulis* larvae. Gastropod larvae and broken shells.
Mud	16.0	Mud was taken together with Amphipod larvae that had burrowed into it.	19.2	- - -	20.0	- - -
Diatoms	0.0	- - - - - - - - -	2.0	*Skeletonema* *Leptocylindrus* *Bacteriastrum* *Chaetoceros* sp. *Navicula* *Pleurosigma*	2.0	*Pleurosigma* *Navicula* *Nitzchia* *Asterionella* *Synedra* *Biddulphia*
Algae (Chlorophyta and cynophyta)	0.0	- - - - - - - - - - - - - - -	2.0	*Oscillatoria* *Chlorella* *Tetrasposa* *Treubaria*	1.0	*Thalassiothrix* Oscillatoria *Anabaena* *Chlorella* *Protococcus*
Sand and silt	8.0	- - -	14.0	- - -	9.0	- - -

	Aug. 19888		Sep. 1988		Nov. 1988	
	% age	Food Components	% age	Food Components	% age	Food Components
Animal matter	43.0	Amphipods: *Argissa typica, Phtisica acaudata, Dulichia porrecta, Scina borealis, Stenothoe marina*. Copepods: *Eurytemora, Oithona, Pseudocalanus elongatus, Centropages*. Cumacean: *Dyatylis* sp., *Eurydice* sp., *Cuma* sp. Mysids: *Praunus flexuosus, Sirriella, Parathemisto* sp., *Hyperia galba*.	28.9	Amphipods: *Phtisica acaudata, Ampelisca spinipes, Stenothoe marina*. A large number of cumacean of *Eurydice* sp. Mysids: *Neomysis integer, Mesopodopsis, Sirriella* sp.	31.0	Amphipods: *Phtisica acauda Dulichia porrecta, Argiss typica, Autonoe websteri, Stenothoe marina, Paratyl swammerdomi*. Cumacean: *Eur pulchra, Eurythenes gryll* Mysids: *Sirriella* sp., *Mysidella typica, Gastrosaccus* sp.
Decayed matter	29.5	- - -	32.0	- - -	41.5	- - -
Mollusca	3.0	*Cerithiopsis tuberculatis* shells. *Spiratella* sp. *Janthina exigua*.	8.5	*Mytilus edulis Spiratella* sp.	0.0	- - -
Mud	10.1	- - -	7.0	- - -	10.0	- - -
Diatoms	0.0	- - -	6.0	*Rhizosolenia* sp. *Chaetoceros* sp. *Pleurosigma* sp. *Nitzschia Bacillaria*.	3.5	*Chaetoceros* sp. *Nitzschia Bacillaria, Leptocylindri*
Algae (Chlorophyta and cynophyta)	0.0	- - -	5.0	*Coelosphaerium, Skujaella, Spirogyra, Nostoc, Lyngbya* and Dinoflagellates.	2.0	*Spirulina, Aphanizomenon, Oscillatoria, Spirogyra Skujaella*.
Sand and silt	14.4	- - -	16.6	- - -	12.5	- - -

TABLE 3. PERCENTAGE BY VOLUME OF THE THREE MAJOR FOOD COMPONENTS OF ADULT *CHELON LABROSUS* ANALYSED DURING 1987-1988

Year	Month	Living Organisms Mean	S.D.	Detritus Mean	S.D.	Inorganic Matter Mean	S.D.	No. of Fish
1987	June	47.3	± 7.1	30.5	± 5.9	22.2	± 7.8	22
	July	52.0	± 7.1	20.0	± 6.2	28.0	± 5.0	13
	August	50.8	± 6.5	27.5	± 4.3	21.7	± 4.5	21
	November	33.5	±10.3	46.0	± 8.7	22.5	± 3.8	20
1988	March	30.0	± 6.6	45.0	± 8.1	25.0	± 9.8	35
	April	39.0	± 9.0	40.5	± 7.3	20.5	± 6.6	30
	May	44.6	±10.1	31.4	± 6.8	24.0	±10.0	13
	June	42.3	± 8.4	24.5	± 3.9	33.2	± 9.9	9
	July	44.0	±11.1	27.0	± 8.2	29.0	± 9.9	15
	August	49.5	± 9.9	29.5	± 7.6	21.0	± 8.0	24
	September	39.4	± 8.3	37.0	± 3.0	23.6	± 8.5	24
	November	36.0	±10.5	41.5	± 4.5	22.5	± 5.0	12
Mean:		42.3		34.2		23.6		

TABLE 4. SHOWING THE MONTHLY FREQUENCY (%) IN THE "FEEDING CONDITION" OF ADULT THICK-LIPPED GREY MULLET, *CHELON LABROSUS*

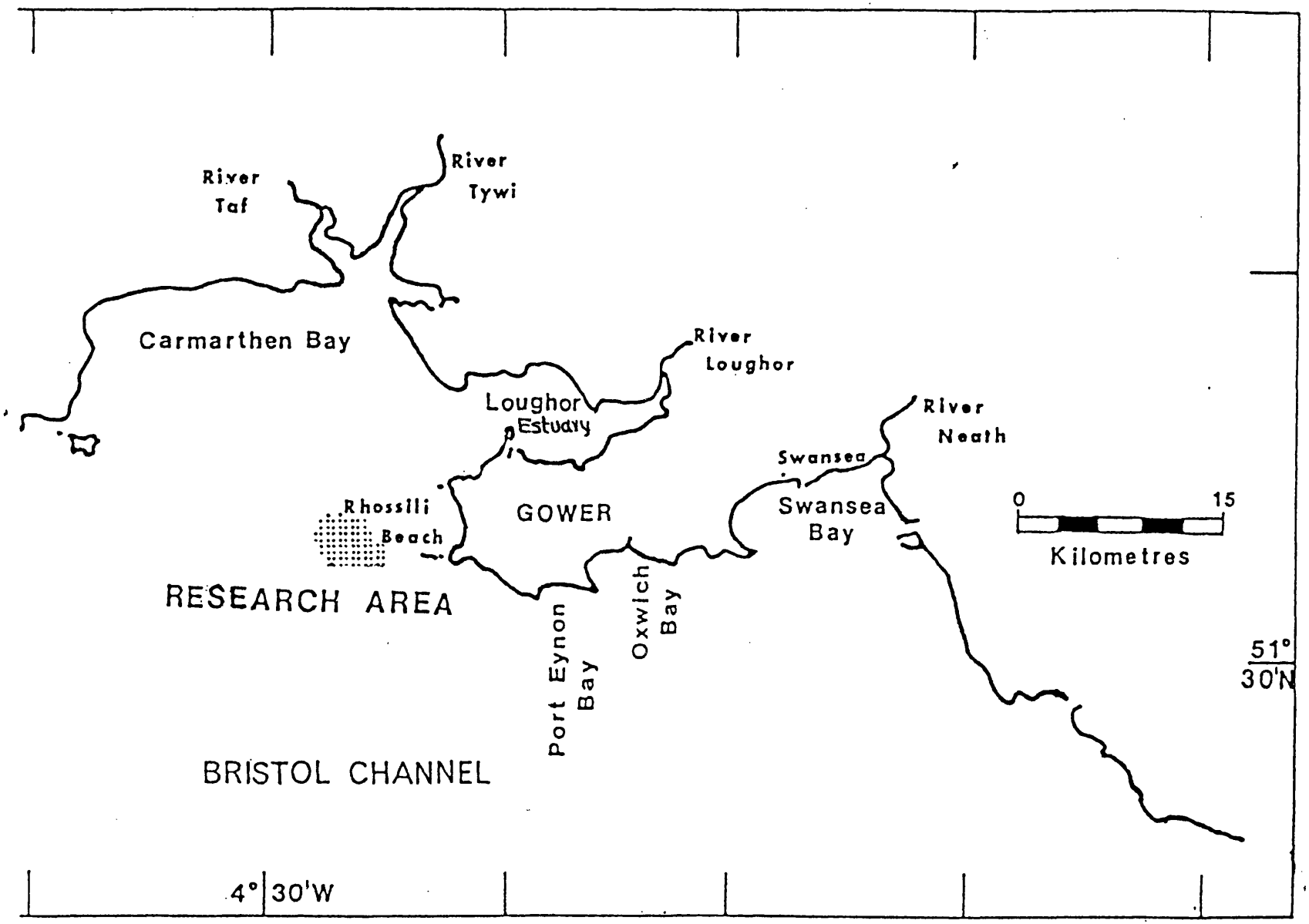
Month	Frequency (%) of Specimens with Stomachs						Total Stomach Examined	Time of Capture
	Gorged	Full	½-Full	¼-Full	Traces	Empty		
Jun. 1987	19.4	25.0	11.1	5.5	11.1	27.7	36	7 a.m.
Jul.	20.0	13.3	26.6	26.6	13.3	0	15	10 a.m.
Aug.	16.1	25.8	12.9	12.9	16.1	16.1	31	3 p.m.
Nov.	18.5	14.8	18.5	22.2	11.1	14.8	27	10 a.m.
Mar. 1988	19.0	28.5	21.4	14.2	9.5	7.1	42	12 a.m.
Apr.	16.6	50.0	23.3	10.0	0	0	30	9 a.m.
May	3.8	19.2	15.3	11.5	26.9	23	26	8 p.m.
Jun.	20.0	40.0	20.0	10.0	10.0	0	10	9 a.m.
Jul.	19.0	28.5	9.5	14.2	9.5	19.0	21	3 p.m.
Aug.	0	0	13.3	24.4	15.5	46.6	45	5 a.m.
Sep.	9.3	18.7	21.8	25.0	12.5	12.5	32	1 p.m.
Nov.	0	3.5	21.4	17.8	14.2	42.8	28	6 a.m.

TABLE 5. THE PERCENTAGE OCCURRENCE OF EACH FOOD ITEM IN THE STOMACH OF ADULT *CHELON LABROSUS*

Month	Jun	Jul	Aug	Nov	Mar	Apr	May	Jun	Jul	Aug	Sep	Nov	% Total Number of
n	26	15	26	23	39	30	20	10	17	29	28	16	Stomachs
Detritus	100.0	100.0	100.0	100.0	100.0	83.3	100.0	100.0	88.2	100.0	100.0	100.0	97.5
Animal Matter	100.0	80.0	100.0	73.9	51.2	70.0	100.0	100.0	100.0	100.0	100.0	100.0	86.7
Mud	100.0	100.0	42.3	39.1	64.1	100.0	50.0	100.0	41.1	20.6	46.8	62.5	61.6
Sand and Silt	100.0	100.0	100.0	78.2	100.0	100.0	75.0	100.0	100.0	100.0	100.0	100.0	96.4
Algae (Diatoms, Chlorophyta and cynophyta)	30.7	46.6	50.0	26.0	25.6	23.3	0.0	40.0	52.9	0.0	28.5	43.7	28.3

n = Number of stomachs.

Figure 1. Map showing the location of Rhossili Bay, the research area in the Bristol Channel, South Wales.


River
Taf
River
Tywi
Carmarthen Bay
River
Loughor
Loughor
Estuary
River
Neath
Swansea
Rhossili
Beach
GOWER
Swansea
Bay
0
15
Kilometres
RESEARCH AREA
Port Eynon
Bay
Oxwich
Bay
51°
30'N
BRISTOL CHANNEL
4°
30'W

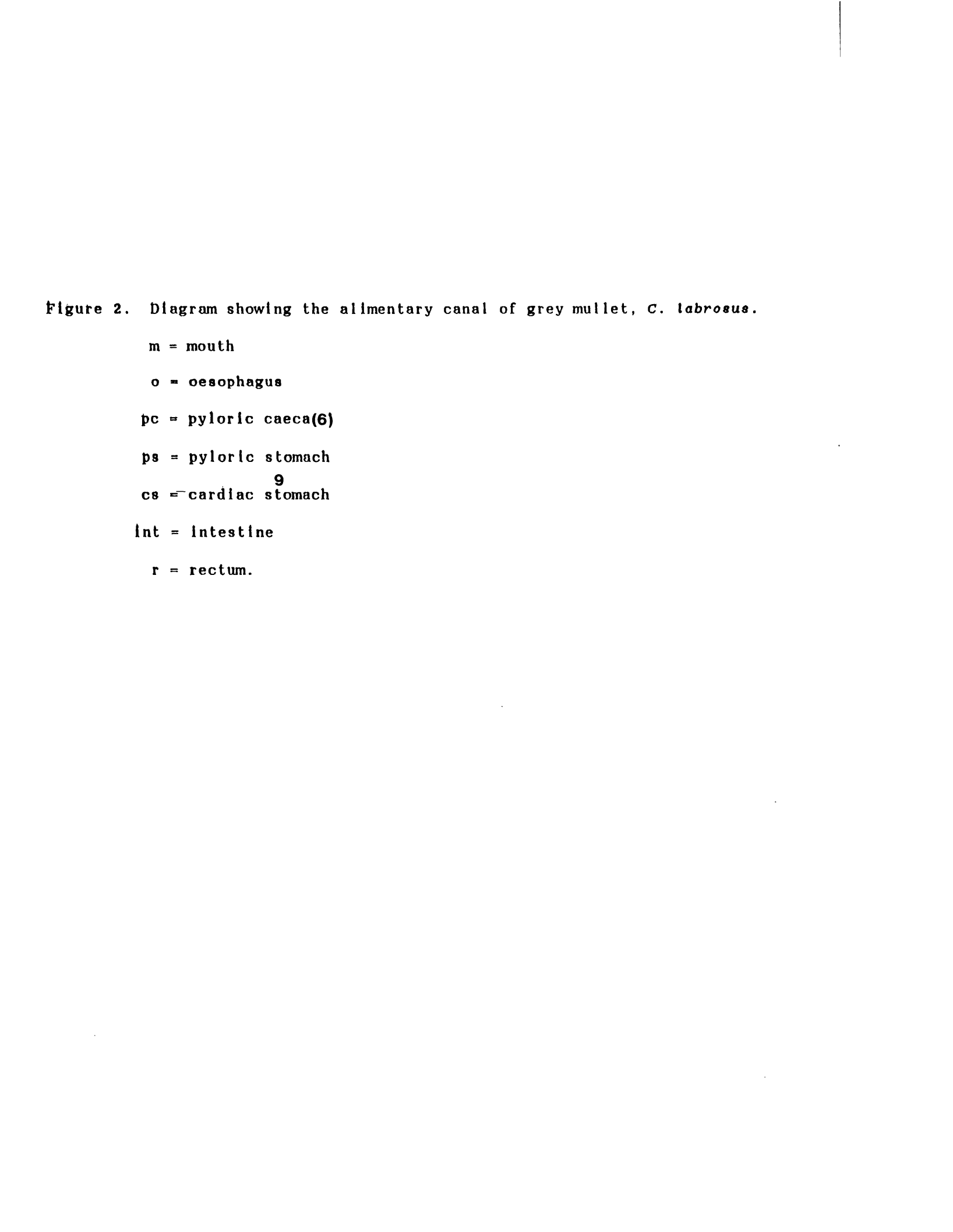


Figure 2. Diagram showing the alimentary canal of grey mullet, *C. labrosus*.

m = mouth

o = oesophagus

pc = pyloric caeca(6)

ps = pyloric stomach

cs = cardiac stomach

int = intestine

r = rectum.

Figure 2.

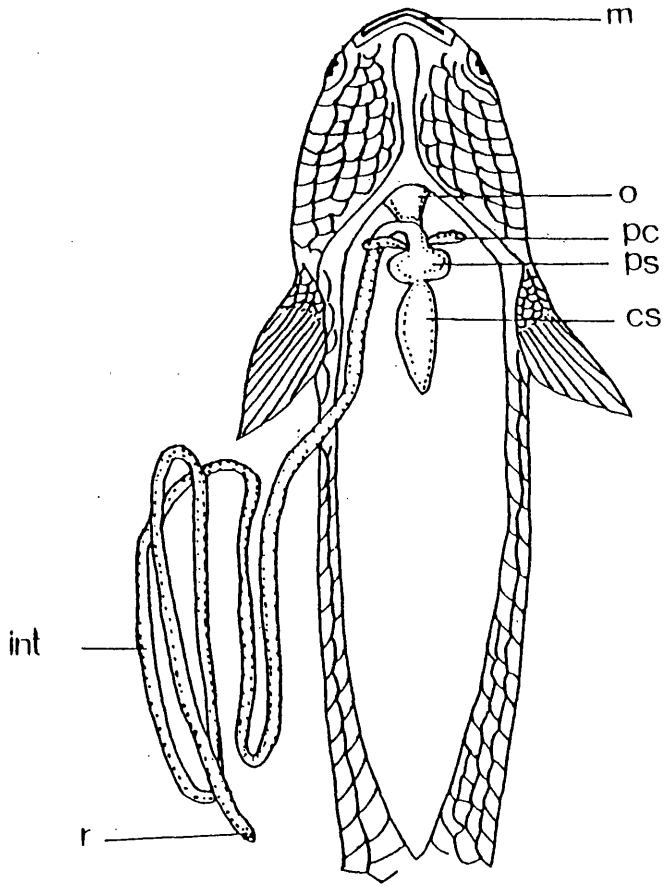


Figure 3. Monthly percentage by volume of the three major food components of adult thick-lipped grey mullet, *C. labrosus*.

Key: Living organisms = Plant and animal organisms

Detritus = Decomposed matter of plant and animal

Inorganic matter = Sand, silt and mud

Figure 3.

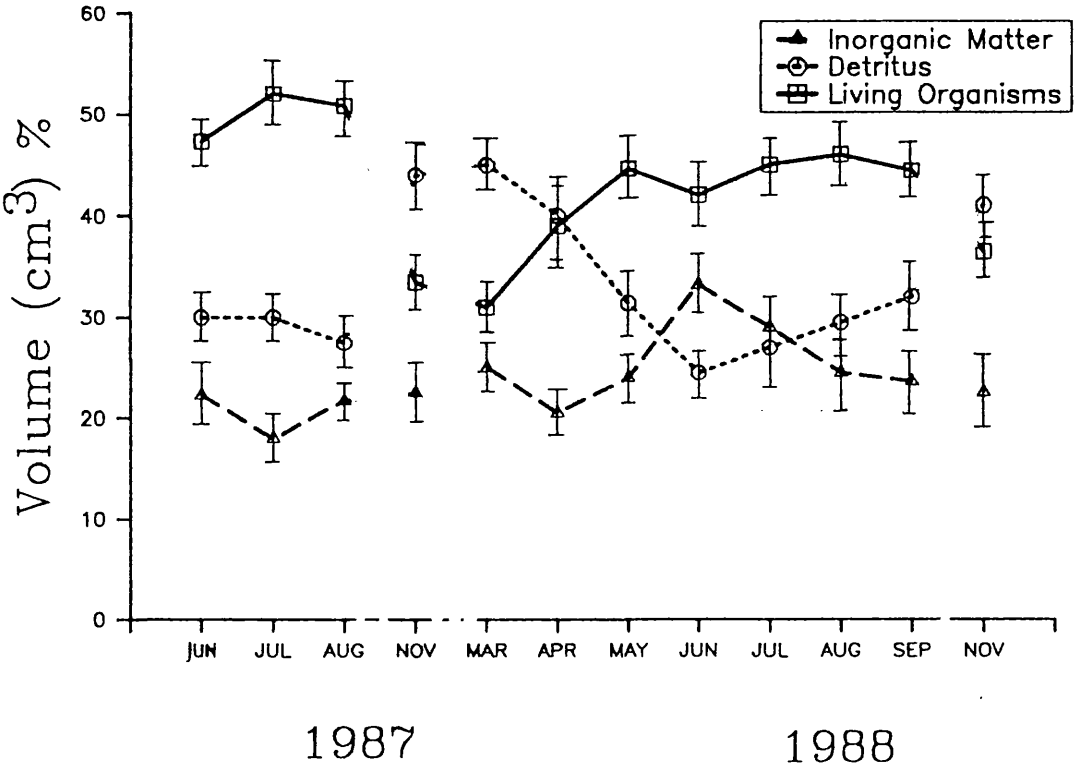
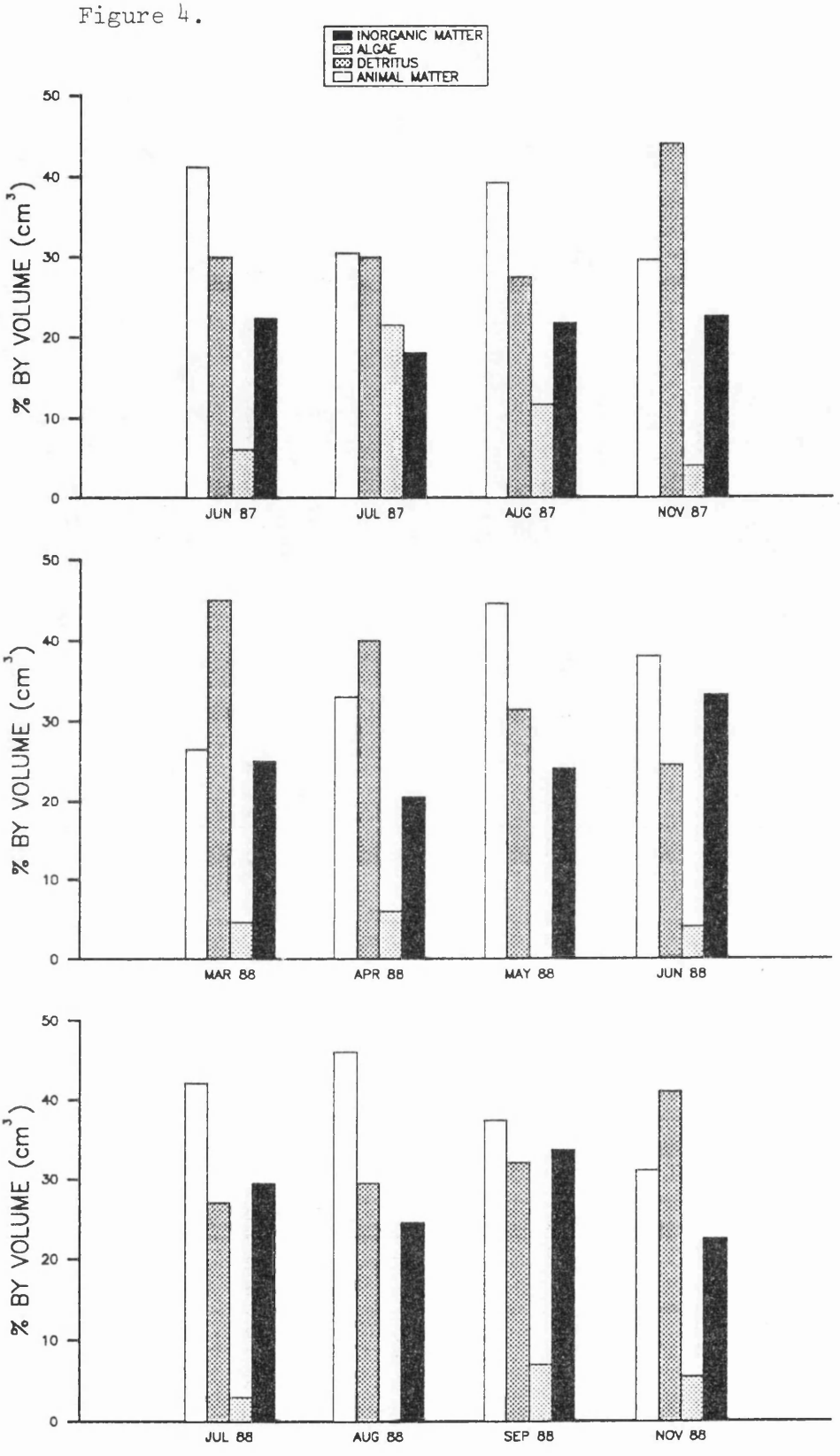

Inorganic Matter
Detritus
Living Organisms
Volume (cm3) %
60
50
40
30
20
10
0
JUN
JUL
AUG
NOV
MAR
APR
MAY
JUN
JUL
AUG
SEP
NOV
1987
1988
Months

Figure 4. Histogram showing the volumetric composition of the food items, animal material, algae, detritus, inorganic matter of adult thick-lipped grey mullet, *C. labrosus*.

Figure 4.


INORGANIC MATTER
ALGAE
DETRITUS
ANIMAL MATTER
% BY VOLUME (cm³)
0
10
20
30
40
50
JUN 87
JUL 87
AUG 87
NOV 87
MAR 88
APR 88
MAY 88
JUN 88
JUL 88
AUG 88
SEP 88
NOV 88

Chapter 3

Population biology of thick-lipped grey mullet, *C. labrosus* and thin-lipped grey mullet, *L. ramada*

INTRODUCTION

The age structure, pattern of growth and the rate of food consumption represent three crucial characteristics of a fish population, which together with its abundance and reproductive output, indicate the ecological status of the population in its environment (Allen and Wootten, 1982). Grey mullet are common fishes in the shallow waters of all tropical and temperate seas. Those of northwestern Europe are mostly found close inshore, and frequently penetrate into estuarine waters (Wheeler, 1969). Three species of grey mullet have been recognised in the English fauna, namely, *Chelon labrosus* (Risso), *Liza ramada* (Risso) and *Liza aurata* (Risso). It is generally accepted that *C. labrosus* and *L. ramada* are the most abundant and widespread of the three species of grey mullet in European waters (Hickling, 1970; Wheeler, 1978). Only *C. labrosus* and *L. ramada* were reported from estuaries in the Bristol Channel (Claridge and Potter, 1985). In British waters, the landing of grey mullet shows that there is a summer fishery and a winter fishery; the former is chiefly in the estuaries when the mullet enter to feed as the water warms up in spring and stay until the water gets cold in winter when they move out of the estuaries to offshore waters (Hickling, 1970). The winter fishery is best known in the west of Cornwall and the Isles of Scilly (Mitchell, 1961) and is based largely on the pre-spawning gathering of mature *C. labrosus* in inshore waters. Inspection of the Sea Fisheries Statistics Tables 1923-79 shows that landings of British grey mullet have increased five-fold over the last 60 years (from 200 cwt to over 1000 cwt). However, it is possible that with appropriate aquaculture this figure could be improved (Anderson, 1982). Hickling (1970) studied the food composition, growth and reproductive biology of grey mullet caught in

the Plymouth estuaries. Kennedy and Fitzmaurice (1969) examined the scales and otoliths of *C. labrosus* from the Irish coast and estimated the age and growth of the fish. Reay (1987) described relative abundance, seasonal occurrence, population structure and growth rate for a population of *L. aurata* from the Hampshire coastal region. Demir (1971) investigated the morphological characters of post larvae and juveniles of *L. aurata* and *C. labrosus*, taken from the Plymouth area. Anderson (1982) gave the key characters for identification of juvenile and adult grey mullets of Britain. Claridge and Potter (1985) provided information on the population of *L. ramada*, showing seasonal and annual abundance, size composition and growth in the Bristol Channel. The present study was conducted to investigate growth in length and weight; length at first maturity, age composition and spawning time of grey mullet, *C. labrosus* and *L. ramada* in the Bristol Channel.

Materials and Methods

A total of 930 thick-lipped grey mullet, *C. labrosus* and thin-lipped grey mullet, *L. ramada* were gutted and weighed and total lengths measured. The total length measurements were taken to the nearest centimetre by measuring from the tip of the snout to the tip of the caudal fin. An electronic balance, accurate to 0.01 gm, was used to weight the fish, the smallest of which were less than 1.0 gm. Four or more scales were removed from under the pectoral fin of each *C. labrosus*. The scales of the pectoral area were clearer than those of the other regions because the surface of the fish was subjected to abrasion. The scales were washed in water, mounted on glass slides and examined under a microprojector in order to count the annuli for age determination. The gonads of females were removed and

weighed in both species and gonadosomatic index calculated separately for each species and used to determine the time of spawning.

A. Length-weight relationship of *C. labrosus* and *L. ramada*

The data of length and weight of juveniles and adults was divided into three groups (i) immature; (ii) male; and (iii) female. The immature specimens of *C. labrosus* were used and the mature fish of each species divided into male and female groups. The data of all groups were arranged by calculating mean length and mean weight at approximately 1.0 cm difference in length.

The basic relationship of weight and length has been described by Le Cren (1951):-

$$w = a\ L^{n}$$

where w is weight, L is length, a is a constant and n an exponent which usually lies at 3.0 if the growth is isometric. However, it can be different for fish from different localities, of different sex or for larval, mature and immature fish (Le Cren, 1951). To obtain the linear regression the values of the weight and length can be expressed by the log-log transformation. So the equation which defines the relationship is as follows:

$$\log_{10} w = \log_{10} a + n \log_{10} L$$

where a is the intercept and n is the slope of the regression. Plotting the log weight on log lengths the points will lie on a straight line if the fish has the same length-weight relationship, but in a case of variation in the relationship the points scatter from the straight line.

Using the VAX 8900 computer a regression was calculated for the logarithm of weight on logarithm of length by the method of least square for each of the fish groups.

B. Condition factor of *C. labrosus*

An important derivative of growth is condition factor or more popularly K factor. In any material body in which, with increasing size, the linear proportions remain constant, weight (if density remains constant) and any bodily linear dimensions are related as in the following expression:-

$$K = 100 \frac{W}{L^3} \quad \text{(Hill, 1936)}$$

where K is condition factor, w is weight and L is length. As thus determined, K will remain constant no matter how large an animal becomes, providing linear proportions (shape) remain constant. A change of weight at a particular length or change of length, without corresponding change in weight, will alter K. Fish populations often display considerable changes in average condition reflecting normal seasonal fluctuations in their metabolic balance and in the pattern of maturation and subsequent release of reproductive products or even the state of fullness of the alimentary canal may influence K (Richer and Merriman, 1945; Weatherly, 1972).

C. Gonadosomatic index (GSI) of *C. labrosus* and *L. ramada*

The term gonadosomatic index expresses the gonad weight as a percentage of the whole body weight. Change in the GSI of fish is a good indication of the reproductive cycle (Htun-Han, 1978). The gonadosomatic index was calculated for females of both species using the formula:

$$GSI = \frac{\text{Gonad weight}}{\text{Whole body weight}} \times 100.$$

D. Length-at-age of *C. labrosus*

To age grey mullets most workers used hard parts other than scales, such as otoliths, opercular bones and spiny rays (Denizci, 1958; Erman, 1959; Hickling, 1970), but Thomson (1966) and Oren (1981) and others discussed the results of those studies and suggested that scales are the most reliable to assess the age of grey mullets.

In teleost scales, there are sclerites or ridges (circuli) forming concentric circles on the upper layer of the scale. When the scale grows, circuli are deposited on its outer layer. The circuli formed in summer are broad and set far apart; in winter, they are narrow and set closely together. The circuli produced by rapid growth in summer occupy a wide zone, whereas those formed during slow growth in winter constitute a narrow zone. The boundary between the crowded circuli and the well spaced circuli is termed the annulus (usual term) or year ring or annual ring. Usually the age of a fish is determined by the number of annuli.

Scales of *C. labrosus* were used to age the fish. The formation of annuli in *C. labrosus* was similar to that reported in the scales of *Mugil cephalus* L. (Devasundram, 1952). The annuli appear by irregularities in the width of circuli which are more visible on the lateral sides than on the anterior part of the scale. These annuli are wider in the middle of the scale than at the apices.

The growth rate of *C. labrosus* was calculated by the Von Bertalanffy equation (Von Bertalanffy, 1938, 1957). Males and females of *C. labrosus* were not separated for growth rate determination because both sexes have the same rate of growth (Kennedy and Fitzmaurice, 1969).

The Von Bertalanffy equation:-

$$Lt = L\alpha\ (1 - e^{-k\ (t - t_o)})$$

where t = time

Lt = length at time t

$L\alpha$ = asymptotic length (is the mean length the fish would reach if they were to grow to a very old age).

k = a growth coefficient or growth rate parameters.

t_o = age at length zero (is the age at which the fish would have been zero length if it had always grown according to the equation, t_o generally has a negative value).

Using the BBC Master 128K (Acorn) Computer, the above growth parameters were calculated. The computer programme was written by S. Walmsley of the School of Biological Sciences, Swansea University. In order to obtain the parameters $L\alpha$ and k from the equation, mean lengths at each age class were needed. The straight lines obtained intersect the 45^o line giving the value of $L\alpha$.

Observations

1. Length-weight relationship of *C. labrosus* and *L. ramada*

Table 1 shows the summary of regression analysis of lengths and weights. The correlation coefficients for the regression line are approximately 0.995 in all cases indicating a high correlation between the parameters. The regression lines fitted for each group are shown in Figures 7 to 11 and the values of length-weight relationship for all the groups obtained are as follows:-

C. labrosus

Immature: $Log_{10} w = 2.5559$ $Log_{10} L$ -1.3847

Male: $Log_{10} w = 2.4788$ $Log_{10} L$ -1.0972

Female: $Log_{10} w = 2.8117$ $Log_{10} L$ -1.61567

L. ramada

Male: Log w = 2.2376 Log L -0.6934

Female Log w = 2.5472 Log L -1.2232

The regression coefficient shows that females of *C. labrosus* increase in weight in relation to length by 2.81 times while females of *L. ramada* increase in weight in relation to length by 2.54 times. Males of *C. labrosus* and *L. ramada* gain in weight slowly with ratios Of 2.47 and 2.23 respectively. However, in immature fish the increase in weight in relation to length is 2.55 which is less than in females of *C. labrosus* but more than in males of both species.

The increase in weight is slow in relation to length in early stages of all fish but having attained a length of about 11.3 cm, the weight gain is rapid, increasing between 20 to 30 times faster than the length in mature fish.

2. Condition factor of *C. labrosus*

The mean value of the condition factor of mature *C. labrosus* for each month was compared with the mean monthly seawater temperature in Figure 1 and Table 3. The variation in condition factor followed the variation in water temperature which indicated that the condition factor was dependent on water temperature. It reached a peak between June and September but declined during November to April. The maximum condition factor value was obtained in July (1.22) and the minimum (0.77) in February.

3. Gonadosomatic index (GSI) of *C. labrosus* and *L. ramada*

The mean monthly reading of GSI is shown in Tables 6a,b and Figure 2. The monthly GSI values obtained in both species were different. The GSI of *C. labrosus* rapidly declined to a minimum from 4.2 to 0.5%, between April and August. It started to increase in September, was 1.0% and reached 4.2% in November, whereas, the GSI values of *L. ramada* increased from July to September from 1.0 to 5%. The present study does not include readings of GSI during the months December to March because samples were not available in these months, therefore, the exact time of the maximum GSI values was not determined.

The condition of the ovaries indicate that spawning in both species takes several weeks, *C. labrosus* caught in April had spawned recently, though some of them still had a considerable number of ova in their ovaries, but the sample caught in May had spent ovaries. While females of *L. ramada* caught in April and May had spawned long before and their ovaries contained developing oocytes.

The GSI values of *C. labrosus*, declined from April onwards, indicating that spawning occurred during April or May. In *L. ramada*, GSI values started increasing three months earlier than *C. labrosus*, which indicated a difference of about three months in their spawning. *L. ramada* spawn in December to January at the latest.

4. Size of *C. labrosus* at first maturity

All lengths are total lengths. The percentage of mature males and females of *C. labrosus* were calculated at 35, 40, 45, 50 and 55 cm length groups. These are shown in Figure 3 and Table 4. Only 5% males were mature at a length of 37 cm, thirty percent at a length of 40 cm, sixty percent at

45 cm, while all were mature at 50 cm length. Five percent of females were mature at length 40 cm, thirty percent at length 45 cm and all of the females were mature at the length of 50 cm.

5. Sex ratio of *C. labrosus* and *L. ramada*

Because sex could not be determined in juvenile fish, only mature fish were used to determine the sex ratio. The male to female ratio in *C. labrosus* and *L. ramada* was 1:1.1 and 1:1.2, respectively. Monthly variation in the sex ratio is shown in Figures 4 and Table 5. The proportion of females to males in *C. labrosus* increased between June and September ($P<0.01$), and reached a minimum during April and May ($P<0.01$). In *L. ramada* the proportion of females increased between April and August and decreased in September and November ($P<0.01$).

6. Length-at-age of *C. labrosus*

Small specimens of *C. labrosus* were caught at Oldbury Power Station during the first week of December 1987. These specimens ranged in size between 3.8 to 19.5 cm. According to their size they can be divided into two size groups, one with mean length 4.3 cm and another one with mean length of 8.7 cm. Claridge and Potter (1985) also collected specimens of *C. labrosus* from Oldbury and from Pembroke in the late autumn and winter and these specimens ranged in size from 6.1 to 19.7 cm. As *C. labrosus* spawn in British waters during April and May (Demir, 1971), the first recruits arrived in coastal waters by December when they had reached a mean length of 4.3 cm and were still less than one year old (O-group). Another size group ranged in size 8.2 to 10.2 cm in mean length and belonged to the age I-group. The specimens with mean length of more than 10.2 cm were aged by scales. Table 2 shows the mean and the range of length at different age

classes from O-group to XVI-group. The length obtained by Von Bertalanffy equation for each age group is also indicated in Table 2. The growth at age and annual increment in length is shown in Figure 5. The fish grew faster during their first four years with a rate of growth of approximately 6.0 cm per year. Later, the growth rate decreased to between 1.0 to 3.0 cm per year. The Von Bertalanffy growth curve is shown by Figure 6. The values of the growth parameters obtained from the equation were as follows:

$$L_t = 69.5\ (1 - e^{-0.096(t - 0.23)})$$

$$L_\infty = 69.5$$

$$k = 0.096$$

$$t_o = -0.23$$

DISCUSSION

In the present study the regression analyses indicate that in the Bristol Channel the weight of adults and immature fish of both *C. labrosus* and *L. ramada* do not increase in proportion to the cube of the length, the usual value for isometric growth. The length-weight relationship of females of *C. labrosus* and *L. ramada* remains greater than that of males. However, in the English Channel, *C. labrosus* and *L. ramada* have high length-weight relationships and there is no difference in the length-weight relationship between males and females (Hickling, 1970). Farrugio (1975) reported that in immature *C. labrosus* and *L. ramada*, there is a fairly low ratio of length-weight relationship but it increases rapidly when the fish becomes mature for the first time. Erman (1961) and Cassifour (1975) concluded that the length-weight relationship of *C. labrosus* is uniform up to the first or second maturation, but then the length-weight ratio becomes low. In grey mullets, including *C. labrosus*, the length-weight relationship is often

higher for the females than for the males (Morovic, 1957; Erman, 1959; Kennedy and Fitzmaurice, 1969; Das, 1977), however, *Mugil cephalus*, *Mugil parsia* and *Mugil cennesius* do not show any difference in length-weight relationship between males and females.

Estuaries and coastal waters are usually more productive and provide more plentiful food than the open sea. The length-weight relationship of grey mullet living in estuaries is higher than that of the fish living in the open sea (Morovic, 1957; Denizci, 1958). Common grey mullet, *M. cephalus*, in estuaries have a high length-weight relationship but migration to the sea results in a low length-weight relationship, either due to osmotic changes or to changes in food (Ricker and Merriman, 1949; Thomson, 1951; Dinizci, 1958). *C. labrosus* and *L. ramada* leave the estuaries and inshore waters of the Bristol Channel during winter, hence it may be possible that these fish suffer from a food deficiency during migration which may result in low length-weight relationships. Low length-weight relationships of *C. labrosus* and *L. ramada* may, of course, also be influenced by environmental factors, such as temperature and salinity.

The fluctuations in condition factor of *C. labrosus* are closely related to the changes in water temperature as shown in Figure1 . Kennedy and Fitzmaurice (1969) showed that the feeding activity of *C. labrosus* is dependent on water temperature. The fish feed actively above 10^{o}C but cease feeding and become inactive below 8^{o}C. Hickling (1970) and Reay (1987) reported that *C. labrosus* and *L. aurata* had a high reserve of intestinal fat during the summer months from July to October, which indicates that the summertime is the active feeding period for these fishes. Clark (1983) and Brown *et al.* (1989) suggested that the low water temperature influenced the feeding and growth of fish which may result in change of condition factor.

In the present study the condition factor tends to improve progressively throughout the summer and reaches a peak in August, then declines towards the winter months. The seasonal changes in condition factor may be related to environmental factors such as food and temperature.

No direct observations on the spawning of *C. labrosus* and *L. ramada* have been carried out in the Bristol Channel. In the present study the spawning time of *C. labrosus* and *L. ramada* were determined from the variation in GSI values.

According to GSI evidence, *C. labrosus* is a late spring spawner, spawning between April and May and *L. ramada* is a winter spawner, spawning in December. Kennedy and Fitzmaurice (1969) reported that in Irish waters the spawning period of *C. labrosus* lasts several weeks, peak spawning occurring in May. Hickling (1970) and Demir (1971) were of the opinion that in British waters the spawning period of *C. labrosus* is from January to April and that of *L. ramada* in autumn. Claridge and Potter (1985) suggested that in the Bristol Channel the spawning period of *L. ramada* occurs between October and January. In Mediterranean waters, the spawning period of C. *labrosus* is from early December to early April and that of *L. ramada* from early October to late December (Nash and Shehadeh, 1980). According to reports from Mediterranean waters, *M. cephalus* L. has the same spawning time as in *L. ramada* occurring in December and January (Broadhead, 1953; Anderson, 1958; Silva and De Silva, 1981). So the consensus of opinion is that the reports from the different regions of Britain and the Mediterranean agreed with the present study that *C. labrosus* is the spring spawner and *L. ramada* spawns in early winter.

The present study has found that in the Bristol Channel length at first maturity of male *C. labrosus* was 37 cm, 40 cm for female *C. labrosus*.

Hickling (1970) reported that in British waters males and females of *C. labrosus* mature at 35 cm and 38 cm respectively, while Kennedy and Fitzmaurice (1969) gave 38.8 cm as the length of maturity for males and 45 cm for females in *C. labrosus* in Irish waters. In Sri Lanka, males and females of *M. cephalus* L. reach maturity at 34 cm and 31 cm respectively (Silva and De Silva, 1981). In *Mugil dobula* in Australian waters the smallest males with spent gonads were 32 cm and females 35 cm (Thomson, 1957). Jacot (1920) reported that *M. cephalus* first breed at about 43 cm on the Atlantic coast of the United States. Oren (1981) suggested that the age at which grey mullet reach sexual maturity is related to their rate of growth which depends on ecological factors mainly temperature and food supply.

The overall sex ratio of mature male to females of *C. labrosus* and *L. ramada* in the Bristol Channel approximates to 1:1.1 and 1:1.2 respectively. However, this ratio changed with different seasons. The proportion of males of *C. labrosus* increased during the period from April to May and in *L. ramada* from September to November. The reason for this may be spawning migrations in which females migrate earlier and return later. Silva and De Silva (1981) suggested that in *M. cephalus* the increase in the proportion of males could be due to the return of males into the feeding ground after spawning and the females being serial spawners may have to remain longer at sea than the males. Kesteven (1942) found that males predominate in *M. cephalus* catches off Western Australian waters while Sarojini (1957) noted the reverse trend in the reproductive populations of *M. parsia* off the Bengali coast. Hickling (1970) found that females of *C. labrosus* predominate while Erman (1961) and Karvounaris (1963) observed an equal ratio of males to females. Wijeyaratne and Costa (1987) reported that in

Liza tade; males mature earlier than females which may increase the proportion of mature males in the population.

The smallest juveniles of *C. labrosus* collected during the present study were 3.8 cm length during the first week of December. These O-group juveniles reach this length in the period April to December as the fish spawns in April. These recruits attained lengths of up to 8.5 to 10.2 cm in one complete year (I-group). The smallest juveniles of *C. labrosus* caught in British waters by Hickling (1970) were of the same size as in the present study. He found that the fish grow about 6 cm during the first year to become approximately 8 cm long. Kennedy and Fitzmaurice (1969) reported a size range of 4.0 to 5.4 cm for O-group *C. labrosus* in Irish waters. De Silva and Silva (1979) recorded a length of 8.0 cm for *M. cephalus* in the Bosphorus, Turkey. Claridge and Potter (1985) reported that in the Bristol Channel the smallest juvenile of *L. ramada* was 5.3 cm long by December, and that the growth curve of *L. ramada* and *C. labrosus* suggested a similar pattern of early growth in both species. The length of *C. labrosus* at different age classes observed in the present study shows similar growth patterns to those exhibited in the same species in Irish and British waters (Kennedy and Fitzmaurice, 1969; Hickling, 1970). The present study suggests that *C. labrosus* is a slow growing and long lived species as reported by other workers (Kennedy and Fitzmaurice, 1969; Hickling, 1970). However, *C. labrosus* grow faster in the Mediterranean Sea, along the coasts of Morocco and in the Bay of Biscay (Arne, 1938; Erman, 1961; Rossignol, 1951) than the present study, on the English Channel and Irish coasts (Kennedy and Fitzmaurice, 1969; Hickling, 1970). Common grey mullet, *M. cephalus* of warmer waters grow faster compared to *C. labrosus* and *L. ramada* in British waters (Thomson, 1951; Anderson, 1958; Denizci, 1958; Erman, 1959). In

Western Australia the sea mullet, *M. cephalus* can reach mean total lengths of 17.8-22.0 cm in just under a year (Chubb *et al.*, 1981), which corresponds to those attained by *C. labrosus* in the Bristol Channel after three years of life. Clark (1983) observed that the Polar species are generally characterised by slow growth and low metabolic rate as compared with temperate species.

In the present study an asymptotic length of 69.5 cm for *C. labrosus* was calculated using Von Bertalanffy's equation. Oren (1981) estimated the asymptotic length to be 75.5 cm for *C. labrosus* using the data of Hickling from British waters. Karvounaris (1963) and Farrugio (1975) also gave similar figures for asymptotic lengths for *C. labrosus* in Italy and Tunisia, respectively. Thomson (1966) mentioned that in Australia *M. cephalus* attained 68.8 cm asymptotic length. The present study and those of previous studies agreed that grey mullet attained 68.8-74.5 cm asymptotic length, however, growth coefficient varies from region to region.

TABLE 1. SUMMARY OF REGRESSION OF LOG WEIGHT ON LOG LENGTH FOR EACH GROUP OF *C. LABROSUS* AND *L. RAMADA*.

Groups	Length Range (cm)	Weight Range (gm)	No. of Fish	Regression Coefficients (n)	Constants (a)	Correlation Coefficients	Standard Error	Significant F
L. ramada:								
Female	34.8-57.5	464.1-1987.8	150	2.5472	-1.2232	0.9799	0.03539	0.0001
Male	36.0-59.0	547.9-1807.0	200	2.2376	-0.6934	0.9758	0.03272	0.0001
C. labrosus:								
Immature	3.1-25.1	0.7- 163.3	220	2.559	-1.3847	0.9950	0.06436	0.0001
Female	34.0-57.0	559.2-1985.9	190	2.8117	-1.6156	0.9819	0.03493	0.0001
Male	35.5-52.0	669.4-1711.0	170	2.4788	-1.0972	0.9408	0.4473	0.0001

TABLE 2. LENGTH-AT-AGE OF THICK-LIPPED GREY MULLET, *C. LABROSUS*.

Age (Years)	Range of Length (cm)	Observed Mean Length (cm)	Calculated Length by von Bertalanffy Eqn.
0	3.8- 6.3 (10)	4.3	1.48
I	8.5-10.2 (29)	8.7	7.77
II	9.3-13.1 (31)	11.2	13.48
III	11.3-18.2 (16)	15.1	18.65
IV	17.5-25.1 (16)	23.1	23.35
V	19.2-34.0 (13)	29.6	27.62
VI	21.0-37.5 (9)	32.0	35.00
VII	29.0-38.4 (7)	36.2	35.00
VIII	35.5-41.3 (12)	38.6	38.19
IX	38.2-43.2 (10)	41.7	41.08
X	37.0-45.5 (15)	43.2	43.71
XI	39.5-47.2 (11)	45.2	46.09
XII	38.5-51.2 (25)	48.2	48.25
XIII	40.1-51.2 (30)	49.7	50.22
XIV	49.0-53.0 (6)	52.1	52.00
XV	51.0-55.0 (10)	53.5	53.62
XVI	53.5-57.0 (15)	55.5	55.08

( ) Indicates the number of fish.

TABLE 3. MONTHLY CHANGE IN WATER TEMPERATURE AND CONDITION FACTOR OF THICK-LIPPED GREY MULLET, *C. LABROSUS*.

Month	Water Temperature (^{o}C)	Condition Factor	Size of Sample (cm)
1987:			
June	14.0	.99	34-53 (60)
July	16.9	1.21	36-57 (68)
Aug.	17.7	1.1	7-56 (50)
Nov.	10.5	.95	5-59 (75)
1988:			
Feb.	6.9	.7	36-57 (85)
Mar.	7.4	.76	35-55 (102)
Apr.	10.0	.9	35-57 (48)
May	12.9	.94	34-59 (65)
June	15.9	1.14	38-56 (73)
July	18.5	1.22	36-56 (69)
Aug.	18.2	1.13	34-59 (61)
Sep.	16.5	1.16	8-50 (79)
Nov.	9.5	1.11	9-51 (85)

( ) Indicates number of fish.

TABLE 4. PERCENTAGE OF MATURE MALE AND FEMALE OF THICK-LIPPED GREY MULLET, *C. LABROSUS* AT DIFFERENT LENGTH GROUPS.

Length of Fish (cm)	% Male	% Female
3-36 (250)	-	-
37 (108)	5 ± 1.7	-
40 (160)	30 ± 7.3	5 ± 1.8
45 (80)	60 ± 7.5	30 ± 6.2
50 (110)	95.8 ± 3.5	84 ± 10.8
55 (128)	100	100

( ) Indicates number of fish.

TABLE 5. MONTHLY CHANGE IN SEX RATIO OF THICK-KLIPPED GREY MULLET, *C. LABROSUS* AND THIN-LIPPED GREY MULLET, *L. RAMADA*.

Month	No. of Males	No. of Females	Male to Female Ratio
		C. labrosus	
1987:			
June	20	40	1:2
July	25	37	1:1.5
Aug.	33	33	1:1
Nov.	28	31	1:1.1
1988:			
Apr.	33	22	1.5:1
May	102	34	3:1
Jun.	20	31	1:1.5
Jul.	31	56	1:1.8
Aug.	18	26	1:1.4
Sep.	32	64	1:2
Nov.	20	28	1:1.4
Total:	362	402	1:1.1
		L. ramada	
1987:			
June	20	46	1:2.3
July	18	36	1:2
Aug.	21	38	1:1.8
Nov.	48	32	1.5:1
1988:			
Apr.	22	33	1:1.5
May	19	38	1:2
June	30	54	1:1.8
Jul.	25	45	1:1.8
Aug.	26	39	1:1.5
Sep.	48	19	2.5:1
Nov.	42	21	2:1
Total:	319	401	1:1.2

TABLE 6A. MEAN MONTHLY GONADOSOMATIC INDEX VALUES IN FEMALES OF THICK-LIPPED GREY MULLET, *C. LABROSUS*

Year	Mean GSI	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1987:	GSI			0.4	0.3	0.5			3.6	
	n			30	28	31			11	
1988:	GSI	4.2	1.8	0.2	0.5	0.7	1.0		4.2	
	n	12	18	15	22	17	21		16	
	S.D.	1.2	0.98	0.03	0.03	0.02	0.4		0.8	
Mean GSI	1987-88	4.2	1.8	0.3	0.4	0.6	1.0		3.9	

S.D. = Standard Deviation of the mean; n = number of fish.

TABLE 6B. MEAN MONTHLY GONADOSOMATIC INDEX VALUES IN FEMALES OF THIN-LIPPED GREY MULLET, *L. RAMADA*.

Year	Mean GSI	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
1987	GSI			1.0	2.6	3.4				
	n			8	13	6				
1988	GSI	0.7	1.0	1.2	2.6	4.2	5.0			
	n	8	15	6	12	13	20			
	S.D.	0.2	0.3	0.1	0.9	0.6	1.2			
Mean GSI	1987-88	0.7	1.0	1.1	2.6	3.8	5.0			

S.D. = Standard Deviation of the mean; n = number of fish.

Figure 1. Monthly variations in the condition factor and seawater temperature of thick-lipped grey mullet, *C. labrosus* during 1987 to 1988.

Figure 1.

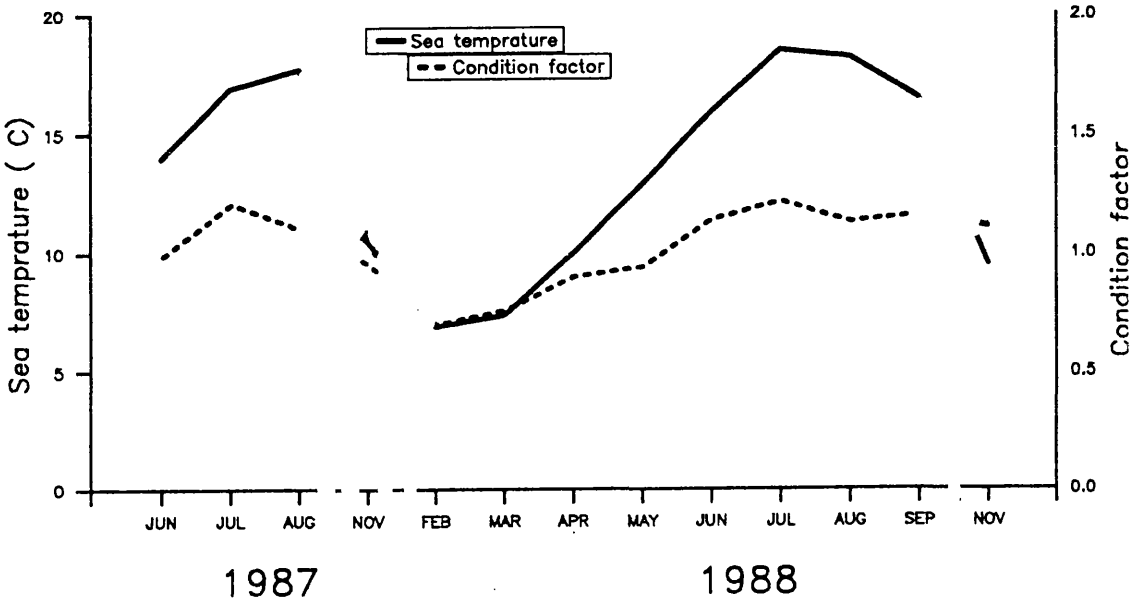

Sea temprature
Condition factor
Sea temprature (C)
Condition factor
20
15
10
5
0
2.0
1.5
1.0
0.5
0.0
JUN
JUL
AUG
NOV
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
NOV
1987
1988

Figure 2. Seasonal changes in the Gonadosomatic Index (GSI) of thick-lipped grey mullet, *C. labrosus* and thin-lipped grey mullet, *L. ramada*. The data for the years 1987 and 1988 are combined.

Figure 2.

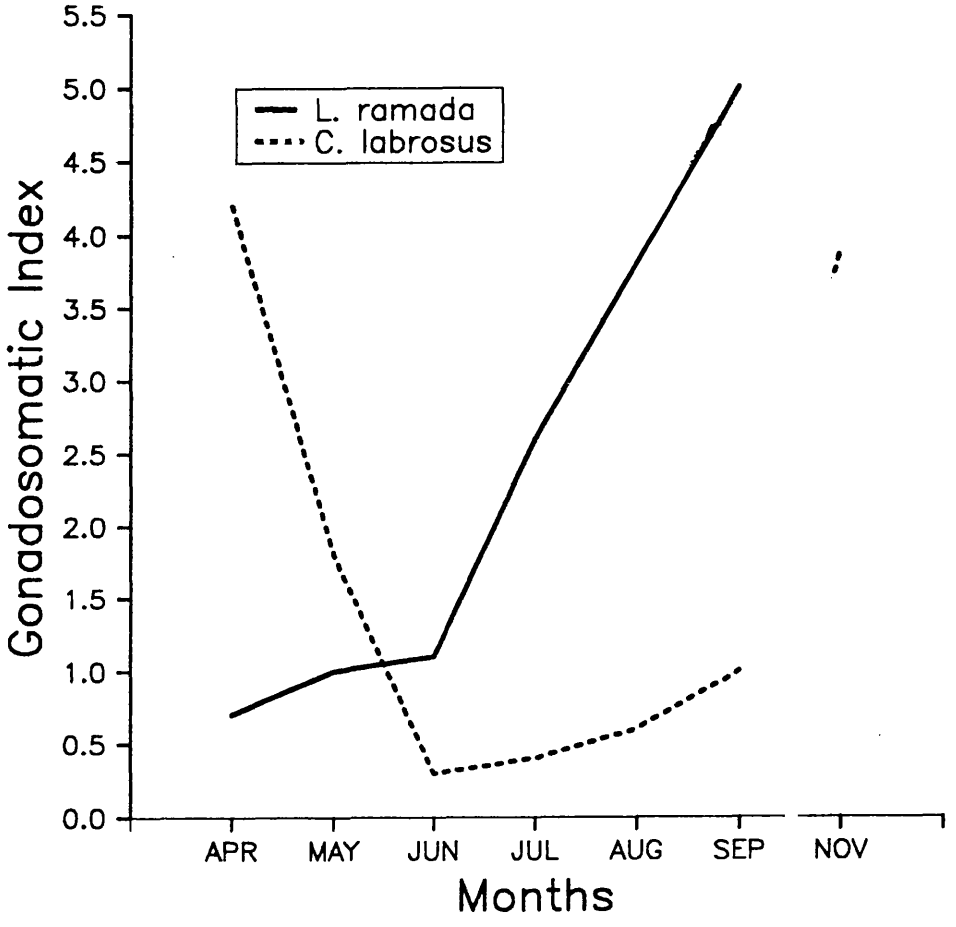

5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Gonadosomatic Index
L. ramada
C. labrosus
APR
MAY
JUN
JUL
AUG
SEP
NOV
Months

Figure 3. Percentage of males and females at first maturity of thick-lipped grey mullet, *C. labrosus* in each 5 cm length difference group.

Figure 3.

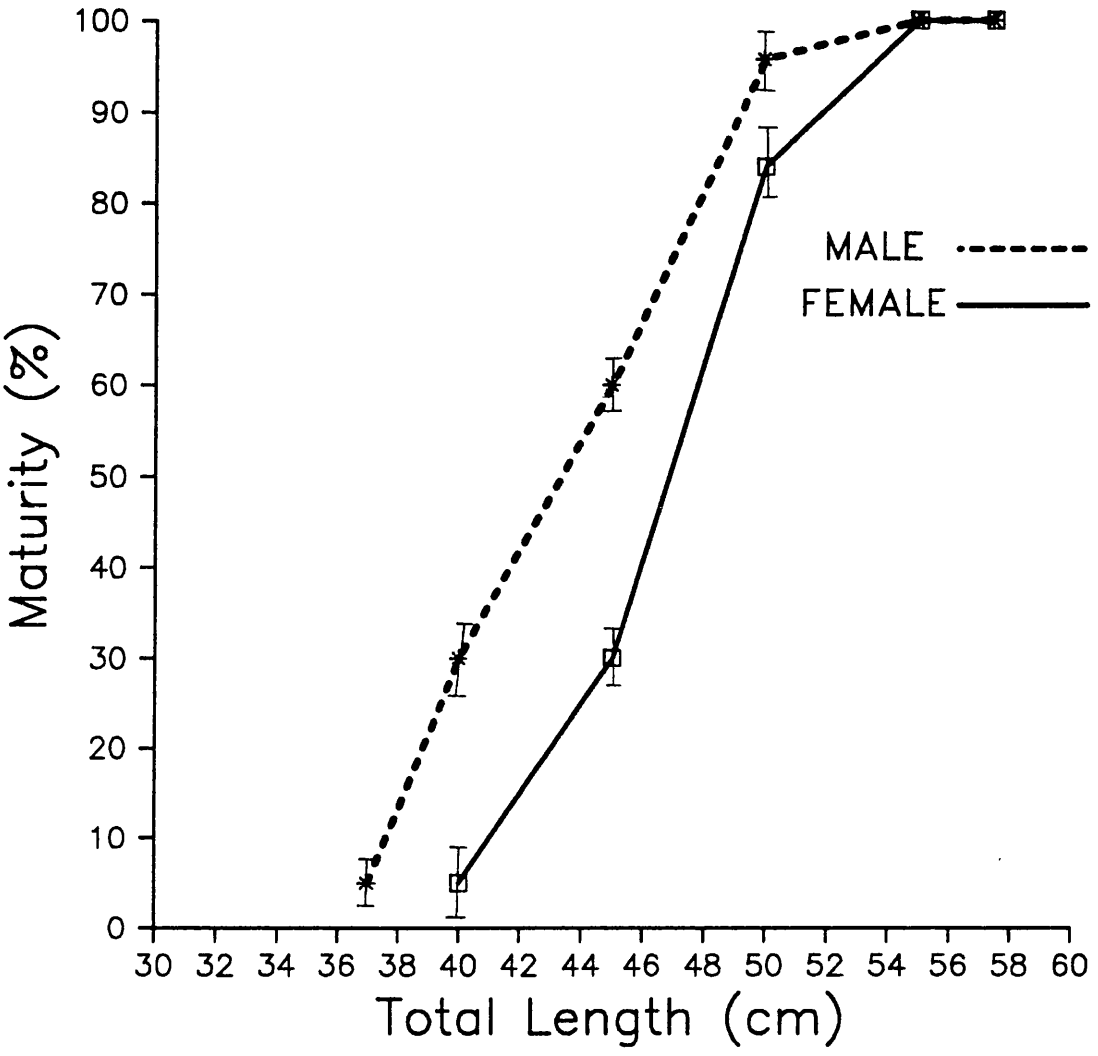

Maturity (%)
100
90
80
70
60
50
40
30
20
10
0
30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
Total Length (cm)
MALE
FEMALE

Figure 4. Monthly variation in female to male ratio of thick-lipped grey mullet, *C. labrosus* and thin-lipped grey mullet, *L. ramada* collected during 1987 to 1988.

Figure 4.

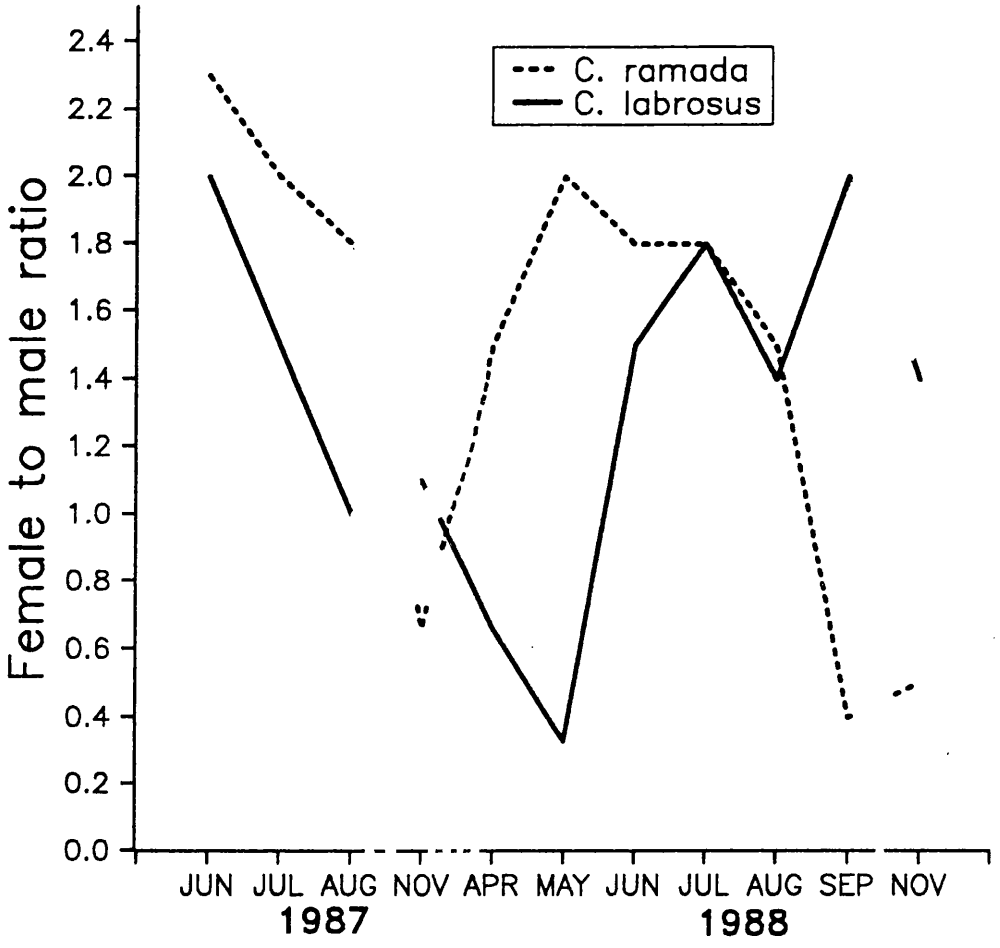

C. ramada
C. labrosus
Female to male ratio
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
JUN JUL AUG NOV APR MAY JUN JUL AUG SEP NOV
1987
1988

Figure 5. Growth in length of thick-lipped grey mullet, *C. labrosus* calculated by von Bertalanffy equation and the annual increment in the length.

Figure 5.

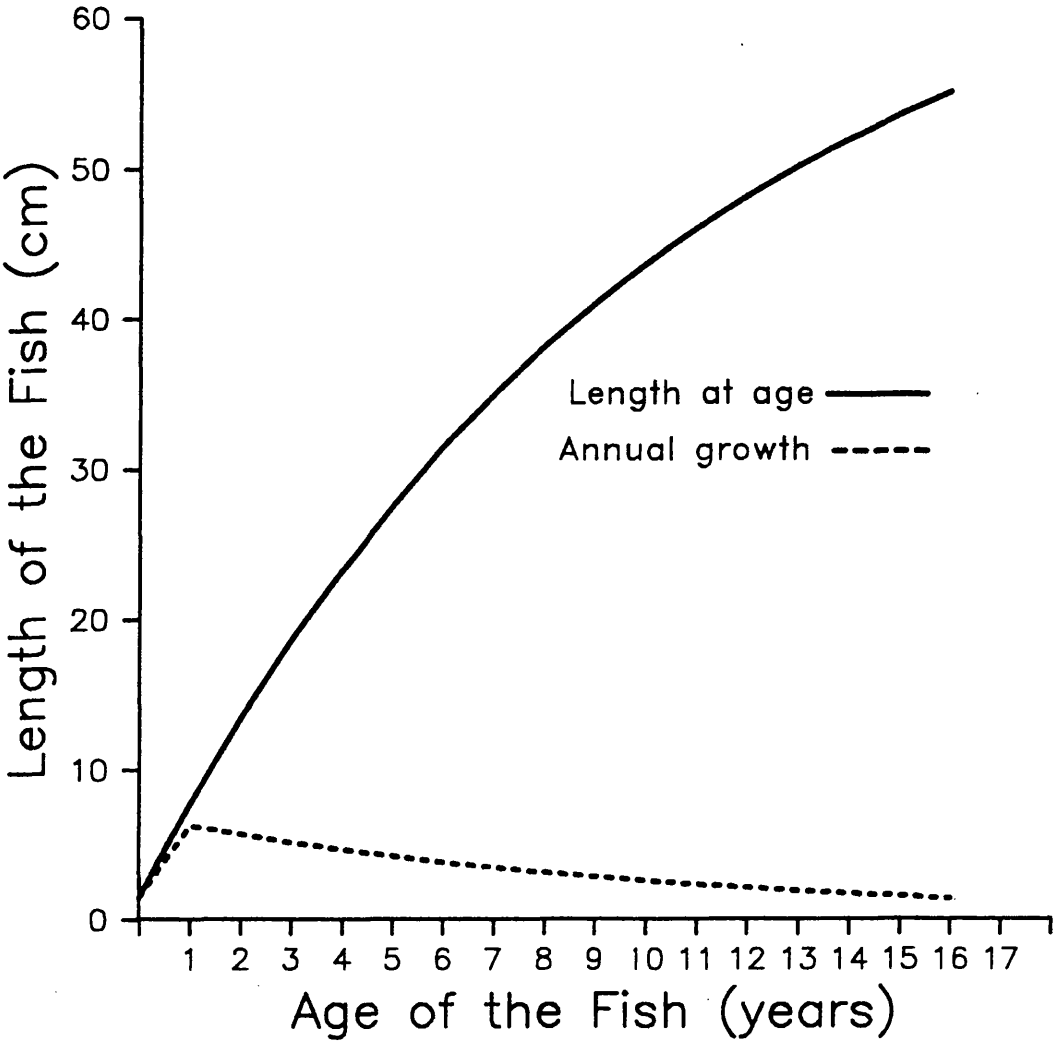
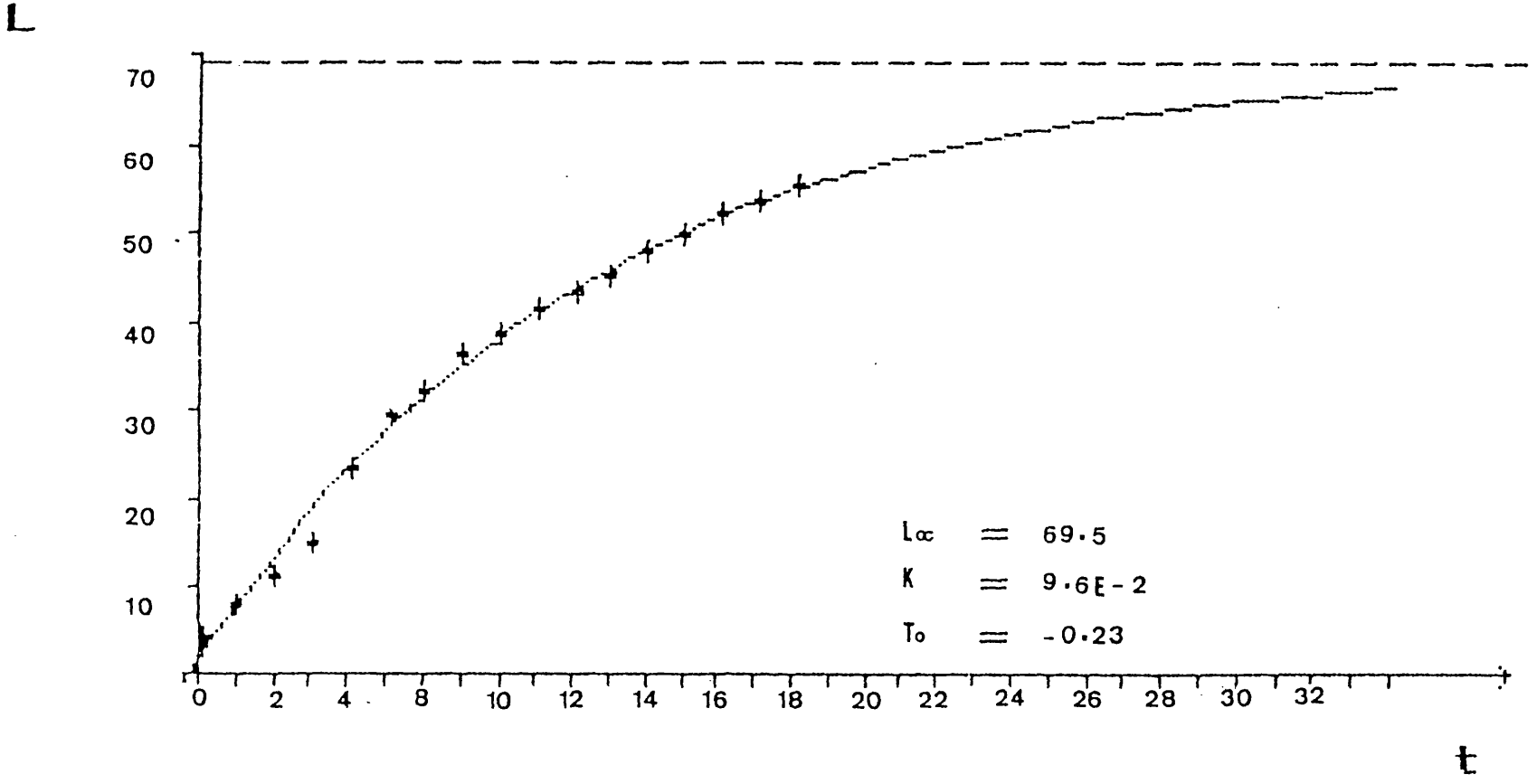

60
50
40
30
20
10
0
Length of the Fish (cm)
Length at age
Annual growth
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Age of the Fish (years)

Figure 6. Von Bertalanffy growth curve (length at age) of thick-lipped grey mullet, *C. labrosus*.

Figure 6.



Von Bertalanffy growth curve

Figure 7. Regression line for the length-weight relationship of immature thick-lipped grey mullet, *C. labrosus*.

Figure 7.

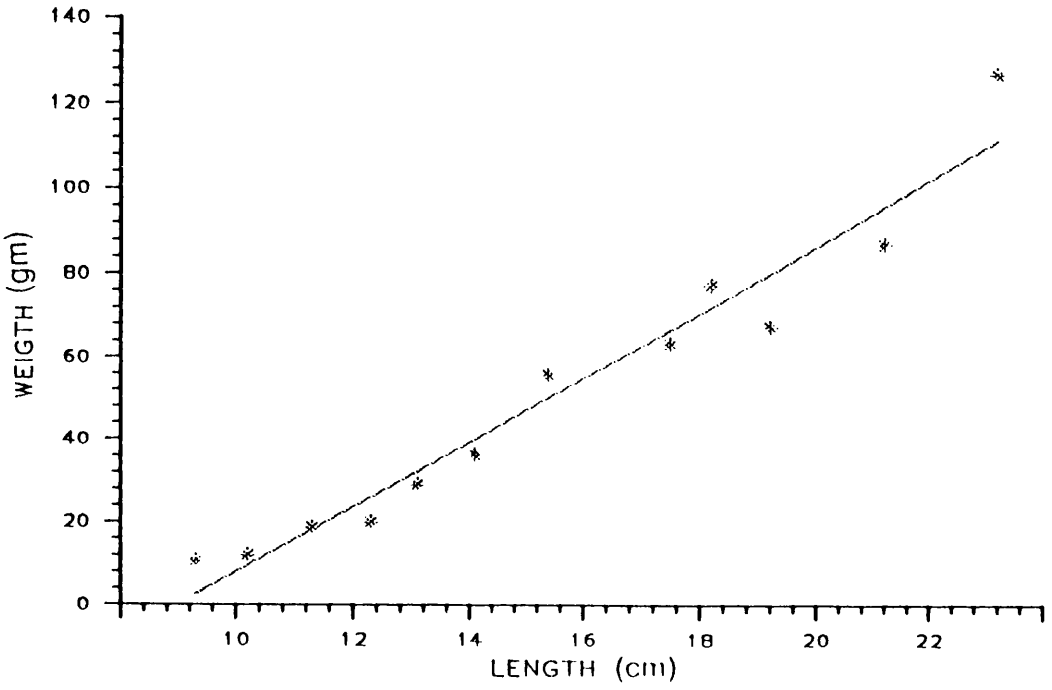


Figure 8. Regression line for the length-weight relationship of female thick-lipped grey mullet, *C. labrosus*.

Figure 9. Regression line for the length-weight relationship of male thick-lipped grey mullet, *C. labrosus*.

Figure 8.

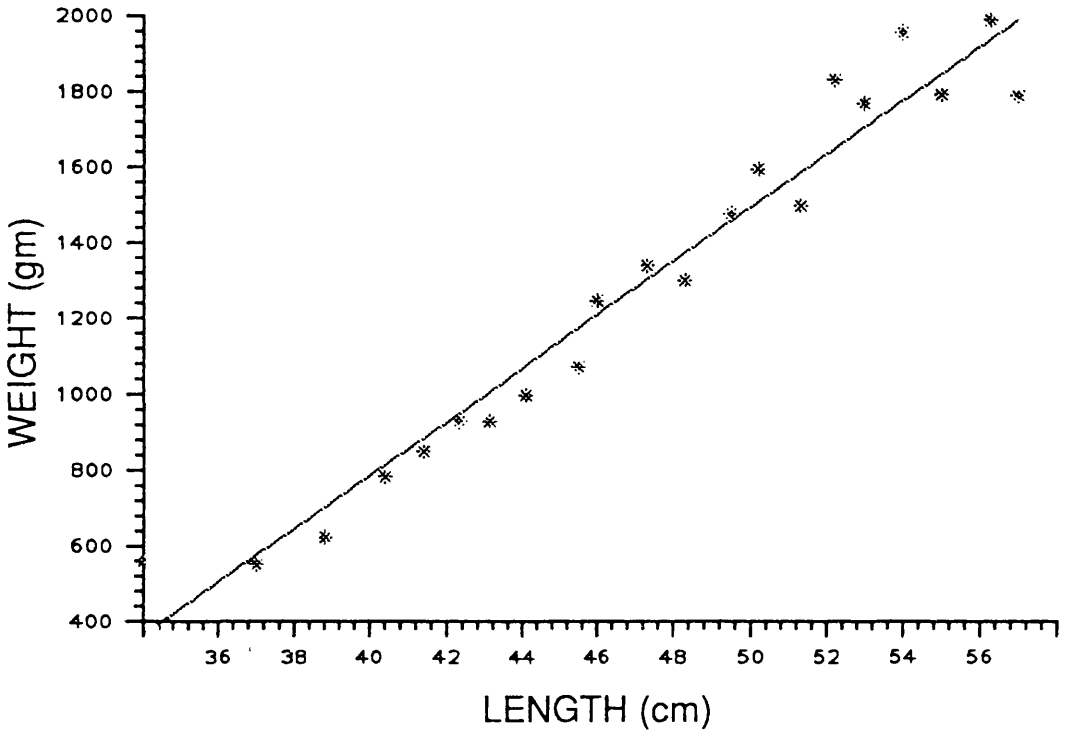


Figure 9.

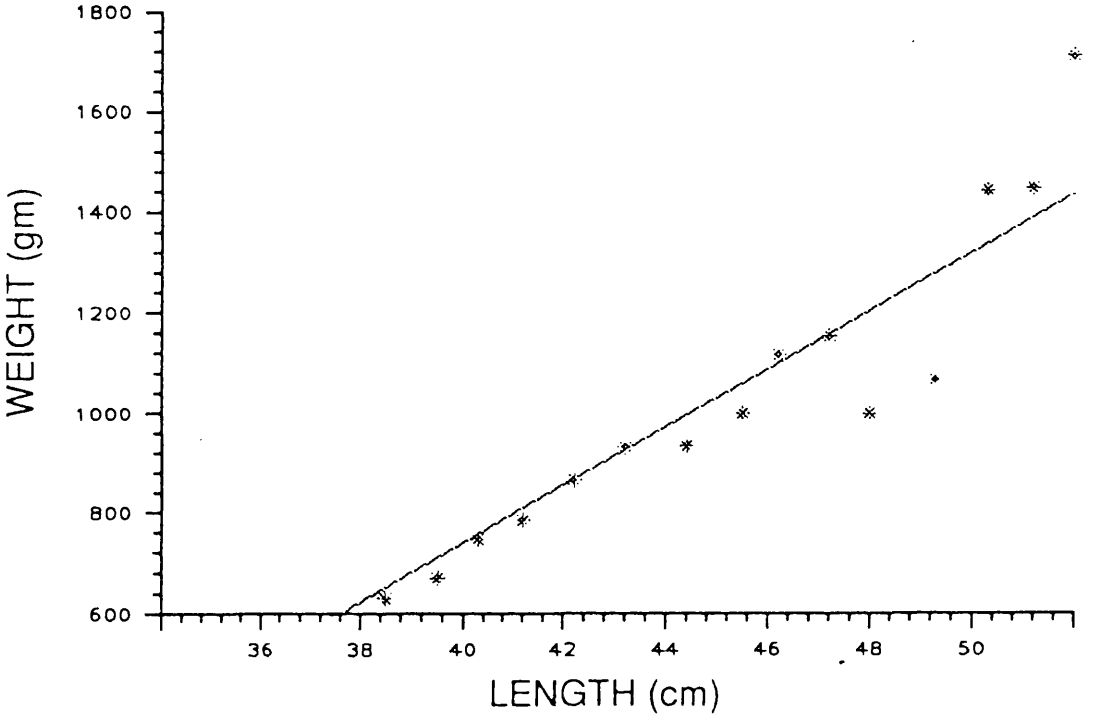


Figure 10. Regression line for the length-weight relationship of female thin-lipped grey mullet, *L. ramada*.

Figure 11. Regression line for the length-weight relationship of male thin-lipped grey mullet, *L. ramada*.

Figure 10.

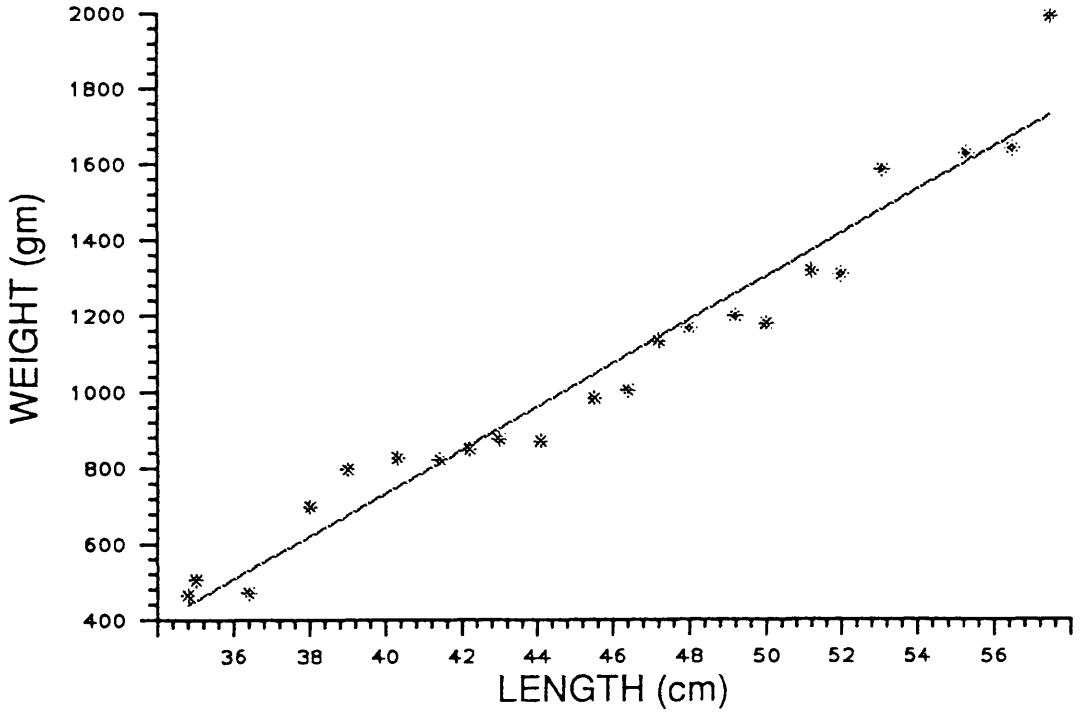
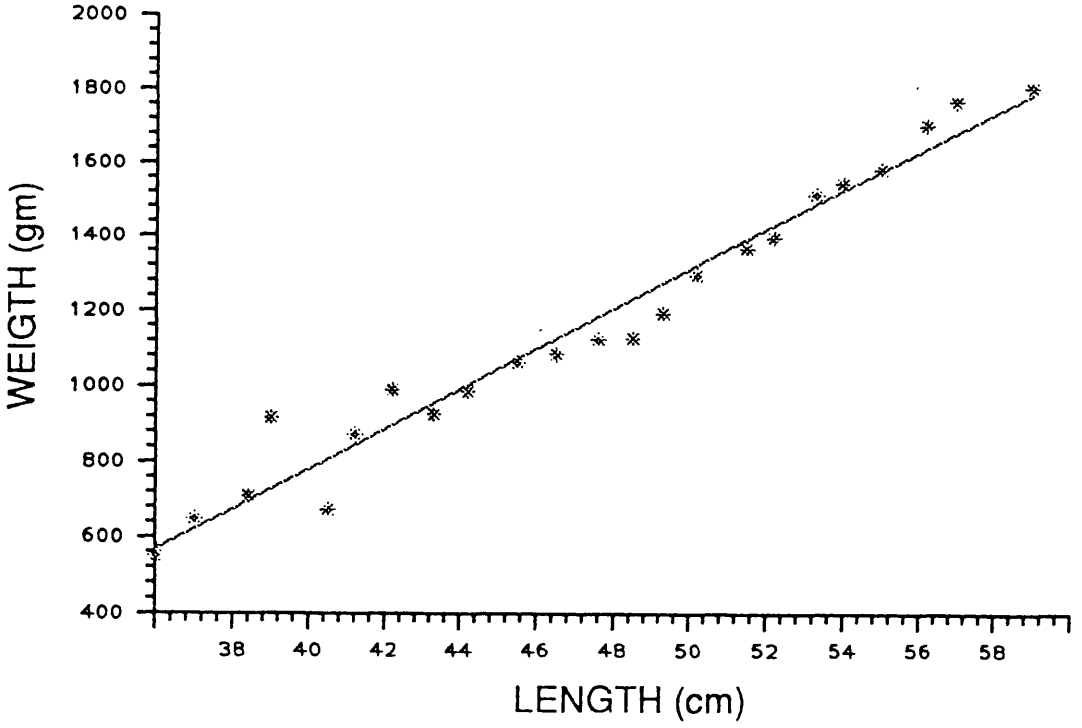


Figure 11.



Chapter 4

Oogenesis in thick-lipped grey mullet, *C. labrosus*

INTRODUCTION

The process of oogenesis is referred to the transformation of oogonia by meiotic division into primary oocytes (Wallace and Selman, 1981; de Vlaming, 1983), but Raven (1961) defined oogenesis as the entire egg development from oogonia through to maturation. This includes the meiotic transformation of oogonia into primary oocytes (oocyte growth, vitellogenesis) and maturation. In this study oogenesis will be used as the later definition. The primary oocyte is surrounded by prefollicular cells, derived from somatic cells, to form a primordial follicle (Moser, 1967). The beginning of folliculogenesis (development of follicle cells) coincides with the initiation of the growth phase at the diplotene stage of the primary oocyte (Wiebe, 1968; Tokarz, 1978).

Two different stages occur during oocyte growth. The primary growth phase in which the size of the primary oocyte increases accompanied by nuclear changes (Barr, 1968; Tokarz, 1978; Khoo, 1979) and the secondary growth phase in which deposition of yolk occurs.

The primary growth phase of the oocyte can be divided into three stages according to nuclear characteristics (Yamamoto, 1956; Yamamoto and Onozato, 1965; Khoo, 1979): the chromatin-nucleolus stage where the oocyte consists of a little cytoplasm and a large centrally located nucleus containing a single, large nucleolus; (ii) the early perinucleolus stage, in which the nucleus is increased in size and nucleoli multiply to increase in number and lie near to the periphery of the nucleus; (iii) the late perinucleolus stage, distinguished by a decrease in the absolute size of the nucleus and a great increase in cytoplasmic volume.

In the perinucleolus stage, most teleost oocytes accumulate an extensive aggregation of basophilic and electron-dense material in the perinuclear cytoplasm (Droller and Roth, 1966; Anderson, 1968; Beams and Kessel, 1973; Guraya, 1979). This electron-dense material is of nuclear origin, passes out through the pores of the nuclear envelope and becomes surrounded by cytoplasmic organelles either mitochondria or a combination of mitochondria, endoplasmic reticulum and golgi bodies (Guraya, 1979; Wallace and Selman, 1981). This structure has been named the 'yolk nucleus' or 'Balbiani body' (Raven, 1961; Norrevong, 1968; Guraya, 1979). This yolk nucleus has been considered as a centre either for the formation of organelles within the oocyte (Guraya, 1979) or for the initiation of lipid yolk synthesis (Nayyar, 1964). As the oocyte grows, the nucleo-cytoplasmic ratio decreases and many membranous organelles are manufactured within the ooplasm (Wallace and Selman, 1981).

With the onset of the secondary growth phase, the follicle cells increase in number by mitosis, to form a continuous follicular epithelium. At this stage the oocyte is surrounded by two follicle cell layers, an outer thecal cell layer and inner granulosa cell layer separated from each other by a distinct acellular basement membrane. The theca layer consists of fibroblast-like cells and is infiltrated by a capillary network. The follicle is believed to be involved in the transport of substances across its membranes (Varma, 1970) or in the formation of steroid hormones (Guraya, 1978; de Vlaming, 1983). Biochemical analysis has revealed that the basement membrane contains mucopolysaccharide and proteins similar to collagen (Guraya, 1978). The function of the basement membrane is still not clearly defined, it may act as a semipermeable barrier between granulosa and theca cells. The oocyte and follicle cells establish an intimate

relationship. The oocyte surface, oolemma, is extended into microvilli and the follicle granulosa cells also extend cytoplasmic processes towards the oocyte. These projections of the follicle cells and the oocyte come into close contact in the intercellular space which is formed as the granulosa cells move away from the oocyte (Wallace and Selman, 1981). Gradually acellular material is deposited between the microvilli of the oocyte adjacent to the oolemma to form the vitelline envelope. The vitelline envelope is also called the chorion, zona radiata or zona pellucida (Laale, 1980). The origin of the zona material has been attributed to the oocyte, to the follicle epithelium or to both (reviewed by Guraya, 1978). Histochemical studies have revealed that the zona material consists of carbohydrates and proteins (Anderson, 1967; Khoo, 1979).

The secondary growth phase is characterised by the formation or accumulation of yolk in the developing oocyte. The process is termed vitellogenesis (Khoo, 1979; Wallace and Selman, 1981). During this stage two processes occur at the same time, namely 'cortical alveoli' formation and 'true vitellogenesis,' i.e., the formation of lipid and protein yolk (Wallace and Selman, 1981). Cortical alveoli (Anderson, 1968; Shackley and King, 1977) or intra-vesicular yolk (Marza *et al.*, 1937) are the first inclusions to appear within the oocyte cytoplasm and contain mucopolysaccharides or glycoproteins (Shackley and King, 1977; Mester *et al.*, 1984) and are believed to be synthesised endogenously (te Heesen, 1977). Cortical alveoli fuse with the oolemma and release their content during the cortical reaction at fertilization (Khoo, 1976) and, thus, are not regarded as yolk inclusions (Wallace and Selman, 1981; Weigand, 1982; de Vlaming, 1983).

The synthesis of lipid yolk precursors has been described as endogenous (forming within the oocyte) and exogenous (forming within the maternal body). Exogenous sources of protein yolk accumulation in the oocyte have been widely described (Anderson, 1974; Shackley and King, 1977, 1978; Weigand, 1982; Mayer *et al.*, 1988), while endogenous contribution to protein yolk synthesis is less common (Droller and Roth, 1966).

Lipid yolk droplets are the first inclusions to accumulate in the developing oocytes and are considered to be the start of endogenous vitellogenesis (Weigand, 1982; Weigand and Idler, 1982; Mayer *et al.*, 1988). Endogenous lipid yolk droplets initially appear in the perinuclear cytoplasm of the developing oocyte (Shackley and King, 1977) and proliferate concomitant with the migration and dispersal of the Balbiani body (Droller and Roth, 1966; Beams and Kessel, 1973). Lipid yolk during early endogenous vitellogenesis consists of phospholipids, later in advanced oocytes it consists primarily of triglycerides.

Protein yolk accumulation commences after lipid yolk accumulation and then proceeds concomitantly. Most yolk proteins appear to be exogenous in origin, the precursors having been identified as the female-specific plasma lipophosphoprotein complex, vitellogenin (Wallace, 1978). Exogenous protein yolk precursors are produced in the maternal body, the liver, and are transported by the blood plasma to the ovary and follicles (Shackley and King, 1977; Idler and Campbell, 1980), where it is taken up by extensive micropinocytotic activity at the oocyte surface (Droller and Roth, 1966; Anderson, 1968; Shackley and King, 1977; Mayer, 1987). Endogenous protein yolk formation occurs by cytoplasmic organelles which are capable of synthesis and packaging the protein yolk granules (Beams and Kessel, 1973; Donato *et al.*, 1980). The size of the oocytes enormously increases due to

the deposition of yolk nutrients reserved for the future embryo (Wallace and Selman, 1981).

After completion of vitellogenesis, the oocyte undergoes maturation. The nuclear membrane breaks up and the contents of the nucleus become mixed with the surrounding cytoplasm. After the breakdown of the nuclear membrane, the chromosomes, which have become greatly contracted and concentrated towards the centre of the germinal vesicle, are carried to the periphery of the oocyte. During meiotic division a cytoplasmic bulge appears at the surface of the oocyte and the outer pole of the spindle with half of the chromosomes enters into this cytoplasmic bulge which pinches off from the oocyte to form the first polar body. The second meiotic division is carried out in the same way, i.e., half of the chromosomes together with a small quantity of cytoplasm are extruded to form a second polar body. The remaining cytoplasm and yolk together with half of the chromosomes is the fully mature egg and can be fertilized (Balinsky, 1981). A rapid increase in size occurs due to hydration during maturation (Kuo *et al.*, 1974). The mature eggs when released from the follicles into the ovary lumen are surrounded by a thick vitelline membrane containing a single opening called the micropyle which facilitates sperm entry at fertilization (Ohta and Takano, 1982; Takano and Ohta, 1982).

Donato and Contini (1974a,b) studied vitellogenic processes in the growing oocyte and the histochemistry of yolk in *Mugil chelo* Cuv. Light microscope studies of oogenesis have been documented in a number of grey mullet species, *M. cephalus* L, *M. saliius* Risso, *M. auratus* Risso, *M. capito* Cuv. and *M. chelo* Cuv. (Abraham, 1963; Abraham *et al.*, 1966; Timosheck and Shilenkova, 1974; Colin and Shehadeh, 1980). An ultrastructural study for cytological identification of germ cells in juvenile and young grey mullet,

M. auratus was carried out by Brusle and Brusle (1978). The present study describes oogenesis in the thick-lipped grey mullet, *Chelon labrosus* Risso at the electron microscopic level.

Materials and Methods

Thick-lipped grey mullet, *C. labrosus*, obtained from local fishermen during March to September 1987-1988, were caught in Rhossili Bay, on the Gower Coast, Wales, U.K. Immediately after capture, the fish were kept on ice and transported to the laboratory as soon as possible. The ovaries of a mature female were removed for either light or electron microscopic study.

For examination with the light microscope, ovaries were fixed in seawater Bouins fixative, dehydrated, embedded in paraffin wax, sectioned at 7 μm and mounted on glass slides. The sections were stained with Mallory's triple stain for general morphological studies (Pearse, 1968).

For electron microscopy, an ovarian lobe was chopped up and fixed for one hour at $4^{o}C$ in 5% glutaraldehyde (TAAB) in 0.1 M HCl-buffered sodium cacodylate with sucrose added, pH 7.2. The total osmolarity for the fixative was 900 milliosmoles. The tissue was then washed in several changes of the buffer solution for 24 hr at $4^{o}C$. The material was dehydrated in a graded series of acetones, mixture of acetone and resin and finally embedded in TAAB resin.

Sections with gold or silver interference colours were obtained using a Huxley Mark I ultramicrotome. Sections were picked up and mounted on copper grids, double stained in 30% uranyl acetate in methanol (15 minutes), followed by lead citrate 6-7 minutes and viewed in a Jeol 1200EX Transmission Electron Microscope.

Observations:-

Stage (1). Oogonia (10-18 μm)

Oogonia are present in the germinal epithelium lining the ovigerous folds of the ovary and are completely enveloped by a layer of follicle cells. They have a centrally located nucleus which occupies most of the cell volume. The nucleoplasm contains dispersed chromatin. The cytoplasm of the theca and granulosa follicle cells contains mitochondria and the cisternae of endoplasmic reticulum (Fig. 1).

In the present study the stages of oocyte development differ from those described in the introduction to this chapter. In *C. labrosus* lipid yolk formation commences in the primary growth phase oocyte and not in the secondary growth phase oocyte as described by other workers (Raven, 1961; Khoo, 1979; Wallace and Selman, 1981). All other characteristics of the oocyte are as defined by Khoo (1979) so it is considered suitable to continue to describe this vitellogenic (lipid) oocyte as a primary growth phase oocyte.

Stage (2). Vitellogenic Stage I (20-110 μm)

This stage can be divided into two stages on the basis of nuclear characteristics.

(a) The chromatin-nucleolus stage:-

The oocytes develop from oogonia by meiotic transformation. They are surrounded by follicle walls which consists of two layers, the inner and the outer layer. The inner layer is called the follicle granulosa, whilst the outer becomes the theca. The granulosa and theca layers are separated by a non-cellular basement membrane (Fig. 2). The follicle layers lie in close association with the oolemma and run parallel to the oolemma.

The ground cytoplasm of the oocyte is homogeneous and contains mitochondria and a confluent channel system of smooth endoplasmic reticulum. Mitochondria are elongate or spherical in shape and are distributed throughout the cytoplasm.

Vitellogenesis commences with the formation of small lipid yolk droplets in the peripheral and perinuclear cytoplasm. The lipid yolk droplets are surrounded by a corona-effect in the cytoplasm and growth of each lipid yolk droplet occurs independently at first, without the fusion of small droplets to form larger. At this stage stacks of smooth endoplasmic reticulum cisternae and mitochondria are present amongst the lipid yolk droplets (Fig. 2). Crenate inclusions appear in the peripheral cytoplasm, which are membrane-bounded, containing granular material in the matrix.

The nucleus is eccentric in position containing a single prominent nucleolus which lies close to the nuclear envelope (Fig. 3).

(b) Perinuclear stage:-

The morphology of the follicle cells is the same as in the previous stage. The oocyte cytoplasm is dense and contains more organelles and inclusions. The mitochondria lie close to the endoplasmic reticulum throughout the cytoplasm and are also concentrated in the perinculear cytoplasm. They are spherical or rod-shaped containing few irregular cristae (Fig. 4).

Endoplasmic reticulum has extensive branches in the periphery and sometimes these branches are continuous throughout the periphery to the perinuclear cytoplasm.

Golgi complexes are present in the vicinity of endoplasmic reticulum and mitochondria. The golgi cisternae lie parallel to each other and small

rounded vesicles elaborate on their tips. These golgi complexes originate from endoplasmic reticulum (Anderson, 1967).

Cortical alveoli appear in the outer cortex. There are two types of cortical alveoli, one is granular and the other is less electron-dense, containing flocculent material. Some stacks of endoplasmic reticulum are present in the surrounding of cortical alveoli (Fig. 4). In the perinuclear cytoplasm small lipid yolk droplets aggregate at the distal part of the endoplasmic reticulum which fuse to become larger (Figs. 4,5). Endoplasmic reticulum appear to be involved in the synthesis or sequestration of the products utilized in the formation of structures and inclusion of cortical alveoli and lipid yolk droplets.

As the oocyte grows the nucleus is reduced in absolute size and the nucleoli increase in number. Each nucleolus contains a fibrous core surrounded by a granular cortex and lies close to the inner side of the nuclear envelope. The budding of nucleolar material towards the nuclear membrane occurs. When vitellogenesis commences the cortical cytoplasm can be subdivided into four zones (Shackley and King, 1977):

(a) Periphery, the zone immediately beneath the oolemma.

(b) Outer cortex, the zone beneath the peripheral zone.

(c) Inner cortex, the perinuclear or innermost part of the cell if the nucleus is not intact.

(d) Mid cortex, the transition zone between the outer and inner cortical zones.

Stage (3). Vitellogenic Stage II (125-200 μm)

With the growth of the oocyte additional follicle cells are added either by mitosis or by attachment of neighbouring prefollicular cells

(Moser, 1967). The nucleus occupies most of the cell area of the follicle cell. The nucleoplasm consists of dispersed chromatin (Fig. 6,7).

The follicle granulosa cells move away from the oolemma, and concomitant with this movement small microvilli originate at the surface of the oolemma and pass into the intercellular space formed as the granulosa cells move from the oolemma (Fig. 6). The granulosa cells also develop small cytoplasmic processes which project towards the oocyte, through the intercellular space and become in close contact with the microvilli of the oolemma. The microvilli and follicular processes are interdigitated with each other, which may facilitate the exchange of nutritive material.

The cytoplasm of the oocyte is relatively dense. The peripheral cytoplasm contains aggregations of elongate mitochondria, rough and smooth endoplasmic reticularum, golgi complexes and vesicles. All these organelles occur in a big cloud (Fig. 9).

Cisternae of endoplasmic reticulum are filled with homogeneous material. The long cisternae break up into stacks and form small vesicles (Figs. 10,12). These vesicles fuse to form cortical alveoli (Figs. 12,13).

Golgi complexes are in an enormous number forming vesicles at their ends. These vesicles consist of homogeneous electron-opaque material, pinch off from the golgi cisternae and lie freely in the cytoplasm (Figs. 6,9,14). The vesicles are either membranous or non-membranous and fuse into each other to form bigger electron-opaque droplets, and may be a precursor of the lipid yolk droplets or represent the premordial cortical alveoli.

The nucleus is eccentric in position and the nucleoli still increase in number. All of the nucleoli are close to the nuclear envelope (Fig. 8). Numerous aggregations of nuclear material are present in perinuclear cytoplasm among mitochondria (Fig. 11). This nuclear material passes through

the nuclear pores. The extruded nuclear material is thought to add to the ribosomal content of the cytoplasm (Kessel, 1966).

Stage (4). Vitellogenic Stage III (200-250 μm)

The intercellular space is widened and filled with homogeneous material which is probably produced by the follicle cell. This electron-dense homogeneous substance deposits in the spaces between the microvilli of the oocyte (Fig. 15). This material has been named the zona radiata, zona pellucida or vitelline membrane. Deposition of the zona radiata is slow (Fig. 19,22). The follicle cell processes are not so numerous as the oocyte microvilli, and project towards the oocyte, traversing the zona material in the pore canals as formed and occupied by the microvilli (Fig. 19).

The ground cytoplasm of the oocyte contains ribosomes and polysomes (Fig. 15). The peripheral cytoplasm consists of golgi complexes, endoplasmic reticulum and mitochondria (Fig. 16). The lipid yolk droplets (Fig. 22) and cortical alveoli present in these aggregations, increase in size and number. Two types of cortical alveoli are present, differing in electron density, one adielectronic and granular and the other less electron-dense containing tubules and vesicles (Fig. 19,20,21). Their size increases by fusion with smaller alveoli. In addition to the presence of lipid yolk droplets and cortical alveoli, for the first time a number of small electron-dense granules of protein yolk appear in the periphery where cytoplasmic organelles are concentrated (Fig. 16). These small protein yolk granules lie freely amongst the stacks of endoplasmic reticulum and also at the distal part of the golgi complexes. At this stage golgi complexes appear to form electron-dense protein yolk granules rather than vesicles. Both endoplasmic reticulum and golgi complexes appear to be involved in the endogenous synthesis of protein yolk granules.

The perinuclear cytoplasm consists of a large mass of mitochondria (Fig. 18,23). This is probably the yolk nucleus (Nayyar, 1964). The number of mitochondria dramatically increase by budding or division and migrate from the perinuclear cytoplasm to the mid cortex (Fig. 17). No other organelle is present in these masses of mitochondria. Small lipid yolk droplets appear in these masses which, by fusion, form bigger droplets. They become more abundant among the perinuclear mitochondria (Fig. 18). The appearance of lipid yolk droplets in the perinuclear masses of mitochondria commence when extrusion of nuclear material occurs. This suggests that both mitochondria and nuclear material together may play an important role in the synthesis of lipid yolk droplets.

Nucleoli are close to nuclear envelope. Aggregations of chromatin material are present in the periphery of the nucleus and similar adielectronic masses occur in the perinuclear cytoplasm. (Fig. 23).

Stage 5. Vitellogenic Stage IV (270-550 μm)

The follicle granulosa cells are cuboidal and contain large prominent nuclei containing chromatin dispersed on the inner side of the nuclear membrane. The cytoplasm contains cytoplasmic organelles including mitochondria, and cisternae of rough endoplasmic reticulum (Figs. 27,28). Several cytoplasmic processes extend towards the oocyte. The intercellular space is very opaque in appearance. The homogeneous material in the intercellular space is probably depositing zona material.

The zona radiata has widened as more zona material is deposited around the pore canals, through which the microvilli pass before protruding into the prominent intercellular space (Fig. 24). The inner layer of zona material has a different electron density to the outer layer and this gives the zona radiata a bipartite appearance (Fig. 24,27). However, with further

oocyte growth a third inner layer of zona material develops adjacent to the oolemma (Fig. 29,30). This scant third layer of zona material is less homogeneous than the two outer layers and has a more organised reticulate appearance. It is derived by chemical change of zona material (Shackley and King, 1977). With the appearance of this inner more organised layer the zona radiata can now be subdivided into two discrete regions, the zona radiata interna and zona radiata externa (Fig. 29,31).

For the first time at this stage, an electron-opaque substance appears at the base of the zona radiata just above the oolemma (Figs. 27,28). This substance accumulates at some places and forms vesicles in the oocyte cytoplasm of irregular shape. These vesicles carry protein yolk granules and pinch off into the peripheral cytoplasm, indicating the commencement of pinocytosis (Fig. 24,25,27,28) and possibly an exogenous source of protein yolk. The peripheral cytoplasm also contains numerous small stacks of rough endoplasmic reticulum, golgi complexes and a few mitochondria (Fig. 27).

The outer and mid-cortex is packed with oval, spherical and elongate mitochondria together with cisternae of endoplasmic reticulum (Fig. 24), these cytoplasmic organelles always occurring in close association with each other. In addition, of these cytoplasmic organelles the outer and mid-cortex contains a number of small aggregations of endogenously formed protein yolk granules which are less electron-dense compared to those deposited by pinocytosis (Fig. 24).

Lipid yolk droplets concentrate in the inner cortex, they become larger by fusion but do not make a continuous mass of lipid yolk. Cortical alveoli also occupy the inner cortex (Fig. 26).

In later stage 5, the follicle cells have enlarged and contain several small electron-dense bodies and electron-opaque vacuoles. The nucleus

consists of a prominent single nucleolus (Fig. 30). Blood capillaries are present in the layer of follicle theca cells. The intercellular space has become occluded and the granulosa cells now lie in close contact with the zona radiata (Fig. 30). Pinocytosis is more active now and the layer of zona material deposited around the microvilli has thickened (Fig. 31,32). The tripartite structure of the zona radiata has become more prominent as the zona interna rapidly increases in width. The zona radiata externa consists of two layers which are more diffuse and show different electron densities (Fig. 31). The highly organised fibrillar structure of the zona radiata interna later become occluded (Fig. 32).

The peripheral cytoplasm immediately beneath the oolemma remains electron-dense and filled with ribosomes, polysomes, storage granules and vesicles (Fig. 29,31). The protein yolk granules increase in number. The small sized protein yolk granules present in the periphery fuse become larger and deposit in the outer cortex (Fig. 29). Some of them are membrane-bounded while others are non-membranous.

The mid- and inner cortex contains sparse numbers of mitochondria and endoplasmic reticulum (Fig. 29,31). Cortical alveoli present in the inner cortex coalesce into large masses and start to move to the peripheral cytoplasm (Fig. 32).

Stage 6. Post Vitellogenic Stage (550-700 μm)

The granulosa cells have enlarged and are in close contact with the basement membrane. The nuclei contain dispersed chromatin. The cytoplasm is packed with spherical and elongate mitochondria, dense bodies and large electron-opaque vacuoles (Fig. 33). Formation of these vacuoles may indicate the degeneration of the follicle cells. The intercellular space becomes occluded and the follicle granulosa cells are in close association

with the zona radiata externa. The tripartite zona radiata is very prominent and has increased in thickness (12-18 μm). The zona radiata interna is approximately 6 times wider than the zona radiata externa (Figs. 34,35). The reticulate structure of the zona radiata becomes occluded and forms a more solid structure. This probably occurs due to deposition of additional zona material. The pore canals are still visible although the oocyte microvilli have been withdrawn from outer parts of the zona radiata (Fig. 34).

The ground cytoplasm of the oocyte is dense containing ribosomes and polysomes (Figs. 37,38,39). The peripheral cytoplasm is occupied by large coalescing cortical alveoli of two types, one being denser and more fibrillar than the other which is less dense with few granules (Fig. 35,36). They occur proximal to the oolemma and at some places have released their contents into the peripheral cytoplasm. The number of cortical alveoli decreases as they fuse and disperse their contents into the area below the microvilli. The vesicles filled with protein yolk granules are still present in the periphery but are now fewer in number (Fig. 34,40).

The outer and mid-cortex contains protein yolk granules, cortical alveoli, numerous mitochondria and rough endoplasmic reticulum (Figs. 34,35,37,38,41). Protein yolk granules coalesce and become bigger but remain as discrete membrane-bound granules (Fig. 34,35,38) still maintaining their structural integrity.

The inner cortex is mostly occupied by lipid yolk droplets and a few protein yolk granules (Fig. 37,42,43). The endogenously formed protein yolk granules are mostly surrounded by lipid yolk droplets (Fig. 42), probably lipoproteins in nature. Mitochondria are less in number compared with other regions of the oocyte.

Stage 7. Maturation (750-900 μm)

The follicle cells are more vacuolated compared with earlier stages (Fig. 44). They contain centrally located nuclei with dispersed chromatin. The nucleoplasm is dense with chromatin. Cytoplasm contains few distorted mitochondria and electron-dense bodies. The zona radiata interna becomes occluded giving the zona radiata a more solid appearance (Fig. 47). The microvilli are withdrawn from a large area of the outer part of the zona radiata. The perivitelline space is prominent at most of the places formed with the elevation of the vitelline envelope (Fig. 45).

The oolemma appears convoluted. Cortical alveoli have prematurely released their contents into the perivitelline space (cortical reaction)(Fig. 48,49). The cortical alveoli with dense and fibriller material form a continuous layer in the outer cortex and move towards the periphery (Fig. 47,50). The peripheral cytoplasm is dense and contains a mass of distorted mitochondria and endoplasmic reticulum but no more vesicles are present in this stage (Fig. 45,47). Protein yolk granules increase in size by further fusion of smaller granules and fill the outer and mid-cortex beneath the cortical alveolar layer (Fig. 46).

The nucleus no longer exists as a discrete entity. The innermost parts of the oocyte are filled with lipid and protein yolk to the exclusion of all cytoplasm and cytoplasmic organelles.

Unfortunately the ultrastructure details of the later stages of maturation and subsequent ovulation were not possible because *C. labrosus* is an offshore spawner and samples were not available.

DISCUSSION

1. Follicle Cells:-

The primary oocyte of *C. labrosus* is surrounded by three walls of follicle epithelium, the granulosa cells, basement membrane and thecal cells. With the growth of the oocyte, follicle cells multiply by mitosis and completely invest the oocyte. During the oocyte development, the morphology of the follicle cells changes, in the primary oocyte they are spindle-shaped and flattened parallel to the surface of the oocyte, but gradually they become cuboidal or columnar. The similar morphological changes were also observed in other teleost species, *Blennius pholis, Dicentrarchus labrax* and *Gadus morhua* (Shackley and King, 1977; Mayer, 1987; Kjesbu and Kryvi, 1989). The follicle cells of the vitellogenic oocytes move away from the oocyte to form an intercellular space and develop cytoplasmic processes which extend into the space towards the oocyte. These follicle processes interdigitate with the ooplasmic microvilli, also formed, which may facilitate micropinocytosis in both oocyte and follicle cells. Hirose (1972) suggested that the follicle cells produce and transport a material necessary for yolk formation in the developing oocyte. The follicle cells are probably involved in the process of vitellogenesis either by transporting or by both transporting and manufacturing the yolk precursors at specific times during the period of vitellogenesis (Shackley and King, 1977).

In the present study during vitellogenesis, follicle cells contain enormous quantities of cytoplasmic organelles such as rough endoplasmic reticulum, golgi complexes and mitochondria. Guraya (1978) suggested that the enrichment of organelles in follicle cells indicate protein synthesis in

these cells. He also suggested that the produced protein in follicle cells may be utilized partly by the cell itself and be partly transported to the oocyte as well, for the formation of the zona radiata. Shackley and King (1977) also reported the presence of concentric whorls of rough endoplasmic reticulum, large mitochondria and large nuclei in follicle cells which indicate the involvement of the cells in an active process or processes of synthesis during vitellogenesis.

The route of protein yolk (vitellogenin) entry into the oocyte have been considered to occur directly through the theca and basement membrane layers from the blood capillaries which supply maternal nutrient to follicle cells. The transport of protein yolk precursors, after these have passed through the basement membrane, may occur through the interfollicular cell spaces (Anderson, 1967; Selman and Wallace, 1982, 1983). Follicle cells are also involved in producing zona material. Shackley and King (1977) reported that the greater part of the zona material is produced by follicle cells in *Blennius pholis* which are possibly manufactured by the columnar granulosa cells. Chambolle *et al.* (1962) also considered the zona material in *Lebistes reticulatus* to be follicular in origin. The follicular origin of part of the zona radiata has been considered to occur in many teleost species (Chinareva and Krichinskaja, 1975; Wourms, 1976; Mohammad-Nagib, 1987; Mayer, 1987). In the present study, the deposition of the zona rdiata after the formation of follicle processes and the numbers of organelles indicate that the follicle cells contribute in producing the zona material at least at the first stage of its formation.

2. Zona radiata:-

The zona radiata is an acellular, electron-dense material deposited in the intercellular space between the oocyte and follicle cells. It is also

called the vitelline membrane, the chorion, zona pellucida or oocyte envelope. In *C. labrosus*, the zone radiata forms at stage 4 (about 200-250 μm) when the spaces between the microvilli become infilled with zona material. As the oocyte grows the microvilli elongate and traverse the zona radiata, passing through distinct pore canals.

The mode of formation and structure of the zona radiata is different among different teleost species. It can be monopartite, bipartite or tripartite in structure (Julli and Julli, 1964; Droller and Roth, 1966; Anderson, 1967; Gotting, 1967, 1976; Wourms, 1976). In *C. labrosus*, the zona radiata is tripartite showing three distinct layers with different electron densities. At first the zona radiata consists of the zona radiata externa consisting of two distinct zones with different electron densities. The reticulate zona radiata is wider, less dense and develops from the interna beneath the zona radiata externa in a later stage of oocyte development. Muller (1962) stated the reticulate network of zona radiata interna in *Cynobellas belotti*, was formed by bundles of fibrils, arranged parallel to the surface of the oocyte in layers at 90^{o}, one to another and with bundles branching around the microvilli so forming the pore canals. Anderson (1967) suggested that the difference in the densities of the zona radiata may reflect differences in the proportion of polysaccharides and proteins contained in each zone. The proteinaceous material is organised into the fibrillar reticulate network by the formation of disulphide bands in *B. pholis* (Shackley and King, 1977).

The origin of the zona radiata is still not clearly understood. Anderson (1967) and Tesoriero (1977) stated that the materials for zona formation are manufactured within the oocyte. In the process, the protein components of the endoplasmic reticulum leave the cisterna in the form of

vesicles, the vesicles fuse with the saccules of the golgi complex when polysaccharide is added. The complex of protein-acid mucopolysaccaride leaves the site of formation at the golgi complex by the vesicles migrating to the periphery where they fuse with the oolemma releasing their contents into the intercellular space. Yamamoto (1953) reported that some evidence such as the appearance of the zona material in close proximity to the oolemma and the increase in thickness by the addition of zona material to the inner surface indicates the oocyte contributes to the formation of the zona radiata. However, the follicular origin of a greater part of the zona radiata is also established in *L. reticulatus, B. pholis* and *D. labrax* (Chambolle *et al.*, 1962; Shackley and King, 1977; Mayer, 1987). In *C. labrosus* the follicle cells contribute to part of the zona radiata certainly in the first stage of its formation by producting homogeneous material in the intercellular space which deposits in between the microvilli. However, the oocyte is the main source of zona radiata interna material which is deposited when the oocyte organelles, particularly the endoplasmic reticulum and golgi complexes are abundant in the periphery.

The functions of the zona radiata have been described as a flexible filter during oocyte development (Zahnd and Porte, 1962; Shackley and King, 1977) or for protection of the embryo during embryonic development (Chambolle *et al.*, 1962). In *C. labrosus*, the zona radiata may play a dual role both as a flexible filter during oogenesis and to protect the developing embryo.

3. *Mitochondria*:-

In *C. labrosus*, the primary oocyte contains mitochondria with irregular cristae. Many of them concentrate in the perinuclear cytoplasm,

whereas others lie in groups associated with endoplasmic reticulum and golgi complex throughout the cytoplasm.

Nuclear material accumulates in the perinuclear cytoplasm and often becomes associated with mitochondria. A similar condition has also been observed in several other studies (Norrevang, 1968; Ulrich, 1969; Shackley and King, 1977; Mohammad-Nagib, 1987; Kjesbu and Kryvi, 1989). Some authors suggest that the nuclear material may stimulate the multiplication of the mitochondria (Balinsky and Davis, 1973; Yamamoto and Onozato, 1965), while others are of the opinion that the mitochondria originate by budding from an elongation and subsequent multiple division of pre-existing mitochondria (Shackley and King, 1977; Mohammad-Nagib, 1987). In *C. labrosus*, it is possible that the mitochondria proliferate by the division of pre-existing mitochondria. However, the nuclear contribution in mitochondrial multiplication can not be neglected.

Mitochondria increase in number as the oocyte grows. In the active vitellogenic oocyte the perinuclear mitochondria enormously increase in number and make a mass movement to the mid- and outer cortex. Small lipid droplets appear amongst these mitochondria which fuse into each other to become larger. Some of the mitochondria contain electron-dense granules in their matrix. These dense granules may be related to calcium deposits (Anderson, 1967; Peachey, 1964). Nayyar (1964) found the "yolk nuclear body" to be composed of lipid and mitochondria and thinks it functions to initiate the synthesis of lipid. According to his observations the yolk nucleus starts migrating from the perinuclear cytoplasm towards the periphery. The lipid droplets in the yolk nucleus grow and slowly move apart and become dispersed in the cytoplasm. When the yolk nucleus reaches the periphery, lipid synthesis stops and the yolk nucleus breaks up and its

components disperse throughout the cytoplasm. The present study agrees with the above findings.

Beams and Kessel (1963) and Shackley and King (1977) reported that, in the mature oocyte, mitochondria are granular and distorted and lie among the protein yolk granules. After the completion of yolk synthesis they shrink but do not disappear. Morphological changes in mitochondria in teleosts have also been observed in several studies. The early oocyte contains large mitochondria but, during active yolk synthesis, they are small, thin and oblong in shape, and increase in number with a typical structure of cristae (Droller and Roth, 1966; Shackley and King, 1977; Mohammad-Nagib, 1987). In the present study mitochondria have no definite shape. In the young oocyte mitochondria are elongate and rounded in shape and some of them contain electron-dense bodies in the matrix; but as the oocyte grows their number increases enormously and they become spherical or oval in shape and are smaller in size. Mitochondria in the mature oocyte are distorted with granular matrix.

4. The Nucleus and the Perinuclear Cytoplasm

The nucleus of the oogonia occupies a large area surrounded by a little cytoplasm (Shackley and King, 1977). In the primary oocyte, the nucleus contains a single prominent nucleolus and is enveloped by a double-layered nuclear membrane containing small pores. During the oocyte development, the nucleus contains numerous spherical and irregularly shaped nucleoli which subsequently take up position close to the nuclear membrane (Anderson, 1968; Forberge, 1982). The granular components of the nucleoli are often associated with the inner lamina of the nuclear envelope. At the outer lamina and in the perinuclear cytoplasm there are patches of dense particles with similar electron density to the granular components of the

nucleoli. Beams and Kessel (1963) mentioned that the small patches or islands of granular material near the nucleoli are the result of a process of shedding and delamination of the irregular surfaced nucleoli. Anderson (1968) suggested that these dense particles are ribosomal and are synthesized within the nucleoli which make their way into the ooplasm through pores in the nuclear envelope. Balinskey (1970) suggested that RNA is transported from the nucleoli into the cytoplasm, where it is to be used in the building of yolk or else to stimulate the multiplication of the mitochondria present in the perinuclear cytoplasm (Yamamoto and Onozato, 1965).

5. Cortical Alveoli:-

In *C. labrosus*, the first appearance of the cortical alveoli coincides with the appearance of lipid yolk droplets and occurs before the formation of protein yolk granules. In *D. labrax*, it was proved by both histological and histochemical tests that cortical alveoli first appear after both lipid yolk and protein yolk formation have started (Mayer *et al.*, 1988), whilst in the majority of teleost species studied, the cortical alveoli appear prior to both lipid and protein yolk formation (Khoo, 1979; Wallace and Selman, 1981; de Vlaming, 1983).

Cortical alveoli are generally believed to be synthesized endogenously (te Haseen and Engels, 1973; te Haseen, 1977). The origin of cortical alveoli have been variously described as vacuolar bodies (Yamamoto, 1961), golgi complexes (Balinsky and Davis, 1963; Gupta and Yamamoto, 1971; Kudo, 1976; Mohammad-Nagib, 1987), endoplasmic reticulum (Shackley and King, 1977) or a combination of endoplasmic reticulum and golgi complexes (Anderson, 1968). In *C. labrosus*, both the endoplasmic reticulum and golgi complexes are involved in the formation of cortical alveoli and cortical alveoli are

therefore endogenous in origin in this species. The first inclusions of cortical alveoli to appear in the oocyte are surrounded by endoplasmic reticulum but later they appear frequently near aggregations of golgi complexes. The golgi complexes produce small vesicles which take the form of cortical alveoli and fuse with each other. Anderson (1968) also observed young cortical alveoli in the pipe fish, *Syngnathus fuscus* and the killifish, *Fundulus heteroclitus*, closely associated with or encircled by flattened cisternae of the endoplasmic reticulum.

In *C. labrosus*, two types of cortical alveoli occur, one is adielectronic and granular and the other is less electron-dense containing flocculent material of tubules and vesicles. Similar types of cortical alveoli have also been reported in other teleost species (Shackley and King, 1977; Iwamatsu and Keino, 1978; Riehl, 1978b). The difference in structure of the two types of alveolus may be due to the uptake of water causing the granules of one type to swell and become the tubules and vesicles of the second type (Shackley and King, 1977).

Cortical alveoli contents are carbohydrates which are released from the oocyte at fertilization (Wallace and Selman, 1981), for this reason they cannot be considered yolk in the strict sense. The discharge of the cortical alveoli at fertilisation is called the cortical reaction (Yamamoto, 1961; Monroy, 1965), and is followed by separation of the chorion from the mature oocyte membrane to form the perivitelline space (Anderson, 1968; Yamamoto, 1961; Kudo, 1976). Other workers suggest that the cortical reaction is not stimulated by fertilization since the release of the alveolar contents and the elevation of the vitelline envelop takes place following ovulation but before spawning and fertilization can occur. In *C.*

labrosus, the cortical alveoli occupy the peripheral cytoplasm in the post-vitellogenic oocyte and they have been observed to fuse with the oolemma and release their contents. Shackley and King (1977) pointed out that their release at the maturation stage could be due to artificial activation by the fixative. This may well be the reason for the premature release of part of the alveolar contents in mature oocytes in the present study.

6. Lipid Yolk:-

The first type of yolk inclusion to accumulate in the developing oocyte is lipid yolk, in the form of small lipid droplets (Raven, 1961; Beams and Kessel, 1973; Shackley and King, 1977, 1979; Mayer *et al.*, 1988). In *C. labrosus*, two sites of appearance of lipid yolk droplets are recognised, the peripheral and the perinuclear cytoplasm. Donato and Contini (1974) reported the appearance of lipid yolk droplets in every region of the ooplasm at the same time in the same species. Mayer *et al.* (1988) reported the first appearance of lipid yolk in *D. labrax* in the mid- and outer cortex, while in other teleosts they generally first appear in the perinuclear cytoplasm (Droller and Roth, 1966; Beams and Kessel, 1973; Shackley and King, 1977; Wiegand, 1982; Mohammad-Nagib, 1987).

Lipid droplets are characterised by a corona-effect surrounding each lipid droplet produced by an organisation of ribosomes in the ground cytoplasm. As the growth of the oocyte proceeds the small lipid droplets fuse to form larger droplets which then migrate to the perinuclear cytoplasm and the corona-effect disappears. This corona-effect around the small lipid droplets has also been found in the guppy, *L. reticulatus* and in the blenny, *B. pholis* (Droller and Roth, 1966; Shackley and King, 1977). The reason for the corona-effect is unknown.

The appearance of lipid yolk has been considered to mark the start of endogenous vitellogenesis (Shackley and King, 1977, 1979; Upadhyay *et al.*, 1978). The endogenous lipid yolk droplets in the early vitellogenic oocyte consist largely of phospholipid (Chopra, 1958; Guraya, 1963, 1965; Nayyar, 1964; Shackley and King, 1977; Weigand and Idler, 1982). After exogenous protein vitellogenesis has commenced, the lipid yolk inclusions consist primarily of triglycerides (Chopra, 1958; Guraya, 1965; Leger *et al.* 1981). This change in composition of lipid yolk is common among vertebrates and invertebrates (Nath, 1960; Raven, 1961). Lipid yolk in *M. chelo* Cuv. is composed of neutral lipid, acid lipid and unsaturated lipids (Donato and Contini, 1974). The endogenous origin of lipid yolk is evident from the capacity of trout ovaries to synthesize triglycerides at a time when vitellogenin uptake is occurring (Weigand and Idler, 1982). The endogenous source has been attributed to cytoplasmic organelles or to occur in the cytoplasm itself (Raven, 1961). In *L. reticulatus*, lipid yolk droplets are usually surrounded by concentric cisternae and vesicles of golgi complexes and it has been suggested that they are formed from the golgi complexes (Droller and Roth, 1966). Nayyar (1964) found the 'yolk nucleus' body in teleosts to be composed of lipid and mitochondria and suggested its function was to initiate the synthesis of lipids. In *C. labrosus* lipid yolk synthesis occurs endogenously. Lipid yolk appears in a cluster of small droplets produced by the endoplasmic reticulum at the first stage of oocyte development. Later, both endoplasmic reticulum and golgi complexes are associated with synthesis of lipid yolk droplets. Small rounded vesicles, always in groups, pinched off from the golgi cisternae together with vesicles form from endoplasmic reticulum fuse to form small lipid yolk droplets. Another active site of lipid yolk formation is the large mass of

mitochondria in the perinuclear cytoplasm, however, the contribution of the mitochondria to lipid yolk formation is not known.

In *C. labrosus*, the small lipid yolk droplets coalesce to form larger masses but they never form continuous lipid yolk masses. Donato and Contini (1974) reported similar observations in the same species. In *B. pholis* and *D. labrax*, lipid yolk droplets fuse to form a continuous mass which fills the areas between the membrane-bounded protein yolk granules (Shackley and King, 1977; Mayer *et al.*, 1988).

7. Protein Yolk:-

Protein yolk synthesis has been studied in many species of teleosts (Wallace, 1978; Shackley and King, 1978; Wallace and Selman, 1981; Campbell and idler, 1976, 1980; Hara *et al.*, 1980). The source of protein yolk precursors are exogenous (outside the oocyte), endogenous (inside the oocyte) or a combination of both which together contribute to yolk formation in the developing oocyte. Exogenous yolk protein precursors are synthesized in the liver under the hormone control (oestrogen) and are then transported by the blood to the ovary where they are taken up by pinocytosis by the developing oocyte (Droller and Roth, 1966; Anderson, 1968; Ulrich, 1969; Campbell and Idler, 1976; Shackley and King, 1978; Yu *et al.*, 1980; Idler and Campbell, 1980; Sundararaj and Nath, 1981). Female-specific proteins, vitellogenin, in teleosts have been detected by immunological, electrophoretic, chromatographic or ultracentrifugal tests in the blood of females during their normal breeding season (Amitante, 1972; Hara, 1975; de Vlaming *et al.*, 1977; Hara and Hirai, 1978; Shackley and King, 1978; Idler *et al.*, 1979; Campbell and Jalabert, 1979; Le Menn, 1979; de Vlaming *et al.*, 1980). In *Brachydanio verio* Ham-Buck, the hepatic origin of yolk proteins

has been confirmed by autoradiographic and electrophoretic studies (Korfsmeier, 1966; te Heesen and Engele, 1973).

Several ultrastructural studies of the oocyte indicate that material incorporated by micropinocytosis is transferred to form protein yolk granules within the peripheral ooplasm (Droller and Roth, 1966; Ulrich, 1969; Gupta and Yamamoto, 1971; Shackley and King, 1977; Wallace and Selman, 1981). Oocytes that accumulate exogenously synthesized yolk proteins are characterised by the presence of numerous pinocytotic bristle-coated pits and vesicles at the cell surface and those vesicles provide the pathway for the selective uptake of micromolecules (Droller and Roth, 1966; Dumont, 1978; Shackley and King, 1977; Selman and Wallace, 1982).

Endogenous synthesis of protein yolk has been described as forming within oocytes under the control of the mitochondria, golgi complexes and endoplasmic reticulum or by a combination of all these organelles. Beams and Kessel (1973) reported that in trout, *Salmo gairdneri*, proteinaceous yolk was synthesized and packaged within the endoplasmic reticulum. In the guppy, *L. reticulatus*, endogenous protein yolk synthesis was first brought about by the golgi complexes. later, vesicles derived from the endoplasmic reticulum coalesced with golgi vesicles to form the protein yolk (Droller and Roth, 1966). Donato and Contini (1974) reported that in *Mugil chelo*, golgi complexes become apparent only in the cytoplasm of the oocyte at the beginning of vitellogenesis and that they are therefore related to the process of protein synthesis. The endogenous contribution to the formation of protein yolk has also been confirmed in *B. verio* (Ulrich, 1969) and salmon (Idler and Ng, 1979), while other studies suggest a combination of exogenous and endogenous synthesis of protein yolk during oocyte development (Droller and Roth, 1966; Ulrich, 1969; Donato *et al.*, 1980).

In the present study protein yolk granules appear after lipid yolk droplets, which is common in many teleosts (Shackley and King, 1977; Mayer *et al.*, 1988). Two sources of protein yolk formation occur, endogenous and exogenous. Endogenous protein yolk granules first appear in stage 4 oocytes (200-250 μm) and are formed by the golgi complexes and the endoplasmic reticulum in the outer and mid-cortex.

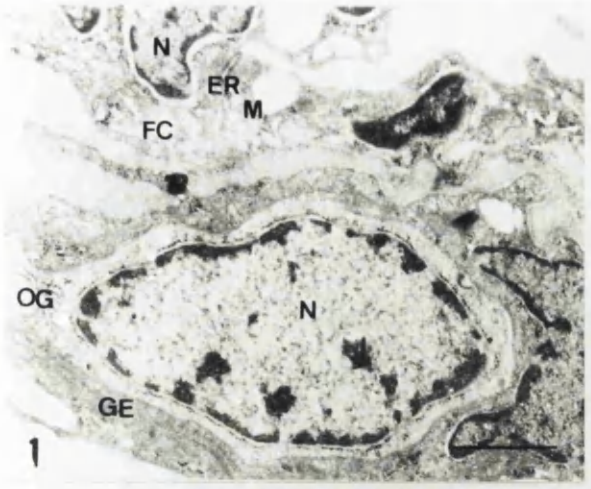
In the stage 5 oocyte (270-550 μm) endogenous protein yolk granules are numerous and increase in size by coalescence. In stage 6 oocytes (550-700 μm) these granules become surrounded by a layer of lipid yolk. The exogenous protein yolk is taken up by pinocytosis at the oolemma. Vesicles form at the base of the oocyte microvilli, budding off into the peripheral cytoplasm. These vesicles carry numerous small electron-dense granules which fuse to become bigger granules but remain separate than those formed by endogenous. Protein yolk accumulations gradually increase in the oocytes which maintain their position in the periphery and outer cortex and occur as discrete membrane-bound granules. The presence of a limiting membrane around the protein yolk granules has also been observed in oocytes of other teleosts (Droller and Roth, 1966; Yamamoto and Oota, 1967; Gupta and Yamamoto, 1971; Shackley and King, 1978). The protein yolk accumulation continues until the stage 6 oocyte (550-700 μm) and the outer and mid-cortex become packed with protein yolk granules. A small number of granules also occur between the lipid yolk droplets in the inner cortex. The exogenous protein yolk granules maintain their structural integrity right through to maturation.

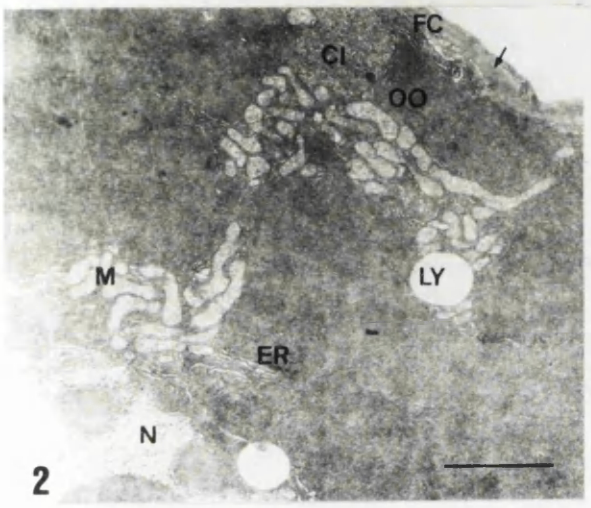
Figure 1. Electron micrograph of oogonia (OG) which occur in germinal epithelium (GE). The oogonia possess central nuclei (N) which contain dispersed chromatin. The prefollicular cells (FC) surround the oogonia and contain mitochondria (M) and endoplasmic reticulum (ER).
Scale bar 500 nm.

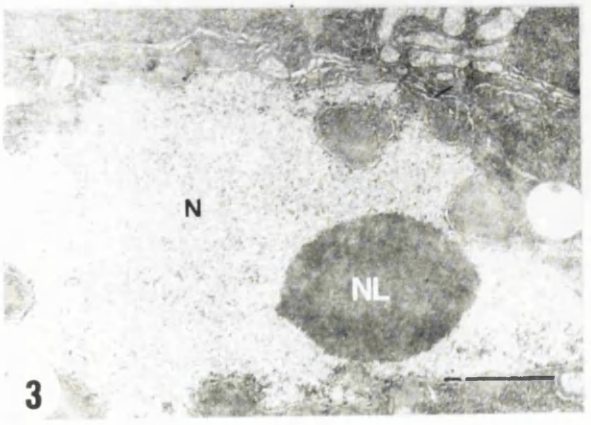
Figure 2. Electron micrograph of a vitellogenic stage 1 oocyte (the chromatin-nucleolar stage). The oocyte is invested by two follicle layers (FC), separated by a basement membrane (arrow). Lipid yolk droplets (LY) are present in the peripheral and perinuclear regions of ooplasm. The ooplasm also contains mitochondria (M), endoplasmic reticulum (ER) and crenate inclusions (CI).
Scale bar 1 μm.

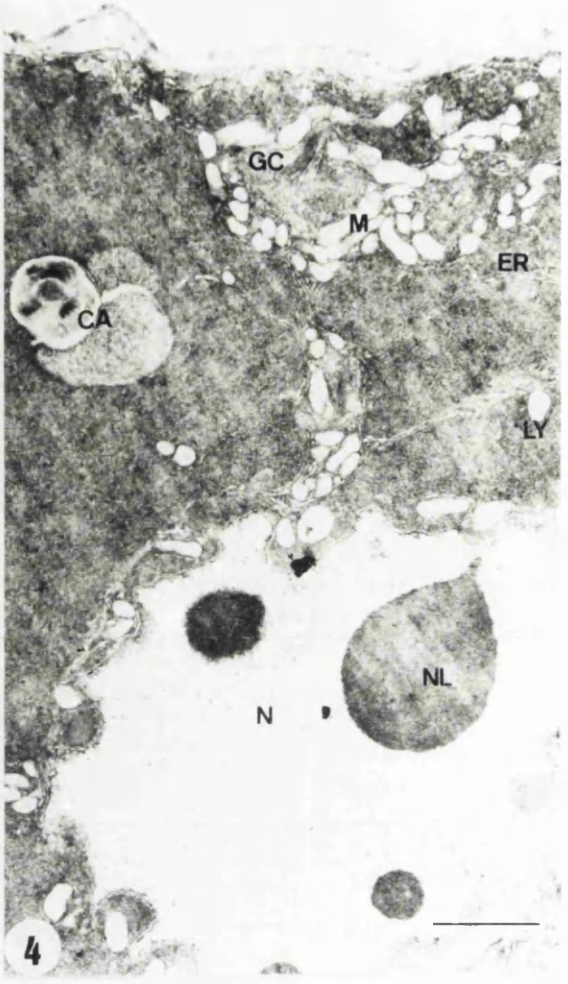
Figure 3. Electron micrograph showing the nucleus (N) of a vitellogenic stage 1 oocyte. The nucleus contains a single nuleolus (NL).
Scale bar 1 μm.

Figure 4 & 5. Electron micrograph of a vitellogenic stage 1 oocyte (the perinuclear stage) showing an increased number of nucleoli (NL). The ooplasm contains golgi complexes (GC), mitochondria (M), and endoplasmic reticulum (ER). Note the appearance of the cortical alveoli (CA) and lipid yolk droplets (LY). Two types of cortical alveoli are present, one is granular and the other contains flocculent material and is less electron-dense.
Scale bar 2 μm.


N
ER
M
FC
OG
N
GE
1


FC
CI
OO
M
LY
ER
N
2


N
NL
3


GC
M
ER
CA
LY
NL
N
4

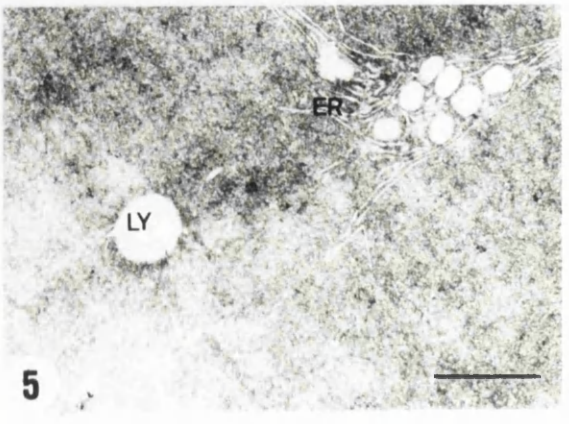

ER
LY
5

Figure 6 & 7. Electron micrograph of a vitellogenic stage 2 oocyte, showing the follicle granulosa cells (FGC) and follicle theca cells (FTC) with large nuclei (N). The oocyte (OO) surface extends into microvilli (MV) which occupy intercellular space. Follicle cells also show cytoplasmic processes extending towards the oocyte. Note the presence of vesicles in the oocyte forming from golgi complexes (GC).
Scale bar 500 nm.

Figure 8. Electron micrograph showing the presence of electron-dense nuclear material (nm) in perinuclear cytoplasm. Note the presence of nucleoli (NL) near the nuclear membrane and dispersion of nucleolar material from the outer edge of the nucleoli.
Scale bar 500 nm.

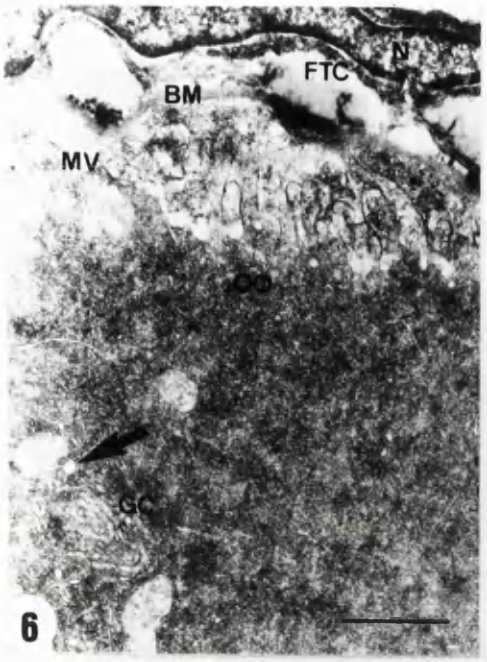
Figure 9. Electron micrograph showing peripheral aggregations of cytoplasmic organelles. Note the presence of vesicles (arrow) and lipid yolk droplets (LY) near golgi complexes (GC) and the enormous number of mitochondria (M).
Scale bar 1 μm.

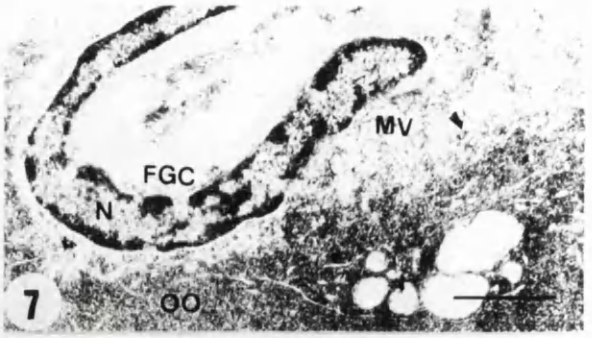
Figure 10. Electron micrograph showing vesicles (arrow) forming from endoplasmic reticulum (ER) and presence of mitochondria in the vicinity.
Scale bar 1 μm.

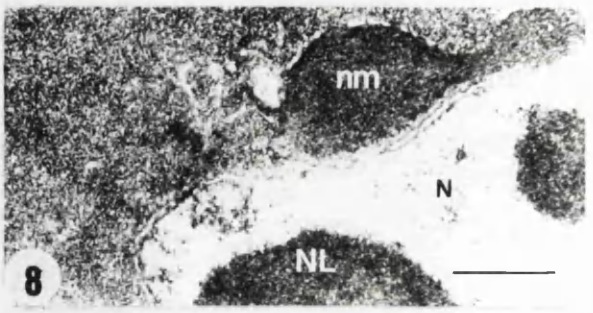
Figure 11. Electron micrograph showing nucleus (N), nucleolus (NL) and the passage of nucleolar material through nuclear pores (arrow). Mitochondria (M) are present in perinuclear cytoplasm among nuclear material.
Scale bar 1 μm.

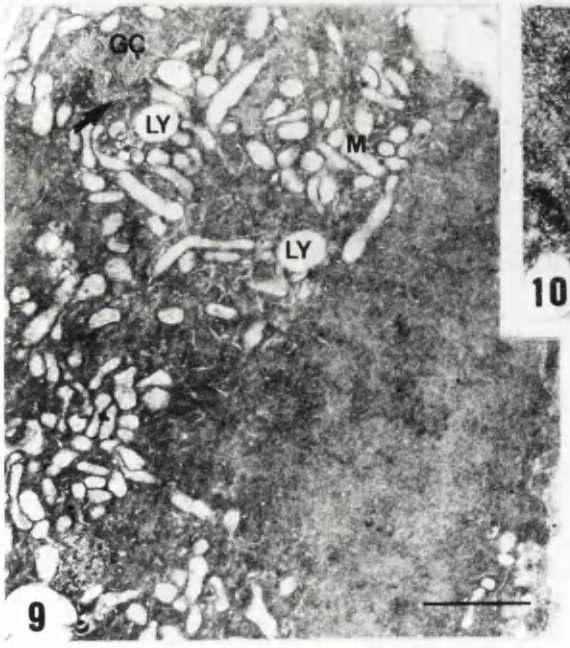
Figure 12 & 13. Electron micrograph showing vesicles (arrow) formed by the break-up of cisternae of endoplasmic reticulum (ER). Cortical alveoli (CA) are present close to these vesicles.
Scale bar 1 μm.

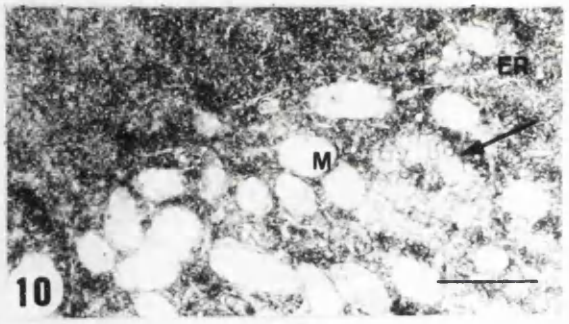
Figure 14. Electron micrograph showing cortical alveoli (CA) and membrane-bound vesicles (arrow) form from golgi complexes (GC). These vesicles fuse to become bigger.
Scale bar 2 μm.

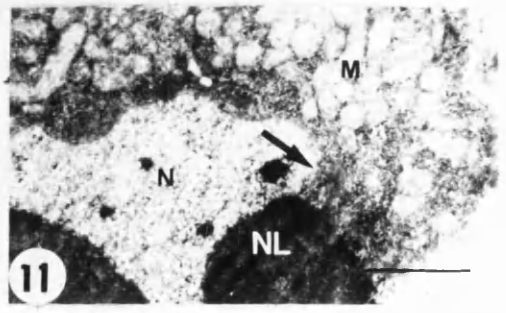

FTC
N
BM
MV
OO
GC
6

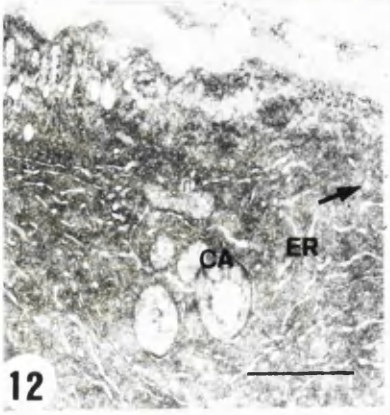

MV
FGC
N
OO
7

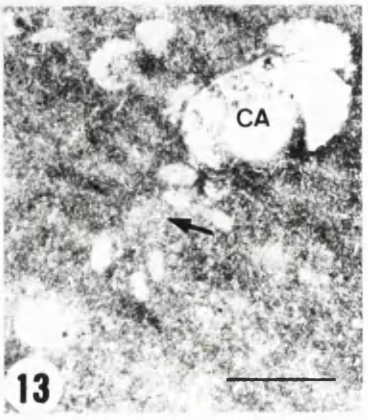

nm
N
NL
8


GC
LY
M
LY
9


ER
M
10


M
N
NL
11


CA
ER
12


CA
13

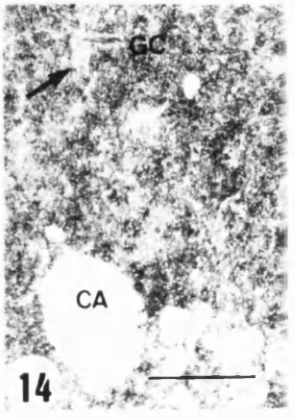

GC
CA
14

Figure 15. Electron micrograph of vitellogenic stage 3 oocyte showing the intercellular space (IS) between the oocyte (OO) and follicle cells (FC), filled with homogenous material. Note the zona radiata (ZR) is laid down between the microvilli (MV) adjacent to the oolemma.
Scale bar 1 μm.

Figure 16. Electron micrograph of the oocyte periphery containing aggregations of mitochondria (M), golgi complexes (GC) and endoplasmic reticulum (ER). Note golgi complexes and endoplasmic reticulum forming small protein yolk granules (arrow). Mitochondria contain electron-dense granules in their matrix.
Scale bar 1 μm.

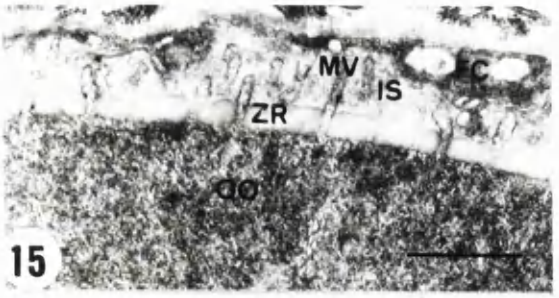
Figure 17. Electron micrograph of the oocyte mid cortex cytoplasm showing a dispersion of mitochondria (M) from perinuclear cytoplasm and presence of lipid yolk droplets (LY) in the mitochondrial aggregations.
Scale bar 2 μm.

Figure 18. Electron micrograph of the oocyte perinuclear cytoplasm showing the presence of several lipid yolk droplets (LY) among mitochondria (M). Mitochondria concentrate close to the nuclear membrane where extrusion of nuclear material from the nucleus (N) occurs. Nucleoli (NL) are present in periphery of the nucleus.
Scale bar 2 μm.

Figure 19, 20 & 21. Electron micrograph of the oocyte periphery showing the cortical alveoli (CA) of two types. They differ in electron-density, one is adieletronic and granular whilst the other is less electron-dense and contains tubules and vesicles. Note the slow deposition of zona material to form the zona radiata (ZR), through which microvilli of oocytes and cytoplasmic processes of follicle granulosa cells (FGC) are passing. BM = basement membrane; FTC = follicle theca cells.
Scale bar 2, 1, 1 μm.

Figure 22. Electron micrograph of the oocyte periphery showing lipid yolk droplets (LY) which become bigger following fusion of small droplets. Note the corona-effect surrounding the lipid yolk droplets produced by ribosomes. ZR = zona radiata.
Scale bar 2 μm.

Figure 23. Electron micrograph of the oocyte perinuclear cytoplasm showing the nuclear material (nm) passing through nuclear pores (arrow) from nucleus (N) to perinuclear cytoplasm. Note the presence of mitochondria (M) and dispersion of nucleolar material (NL).
Scale bar 2 μm.



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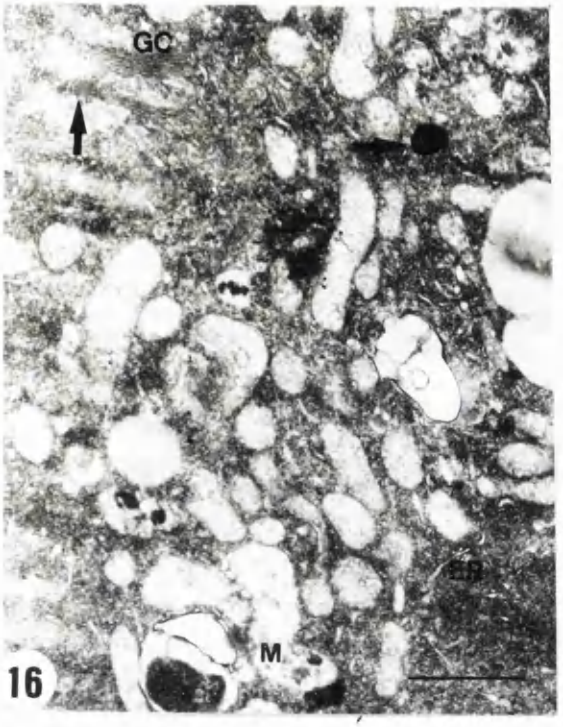

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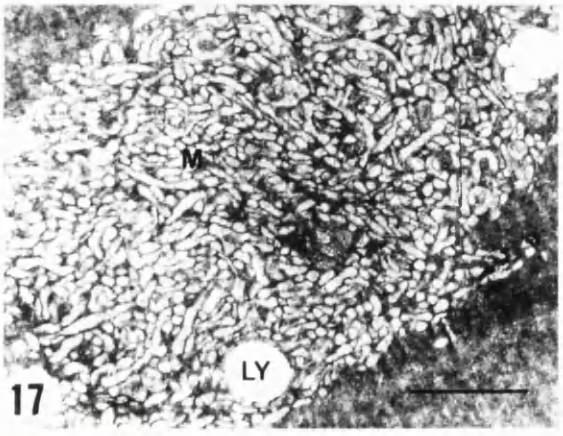

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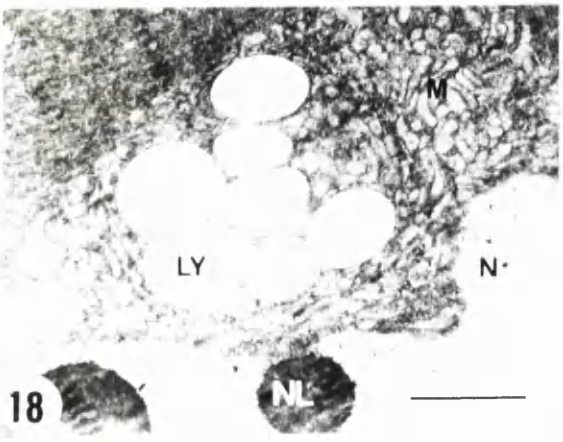

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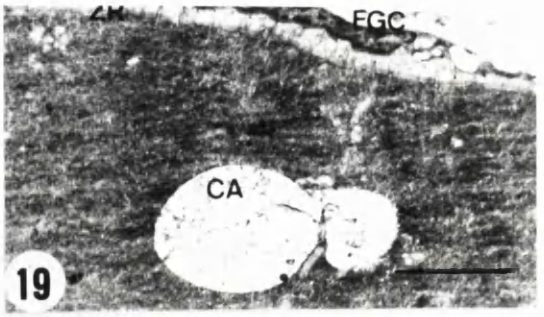

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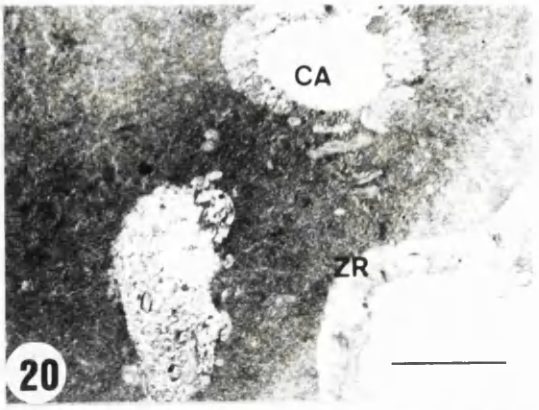

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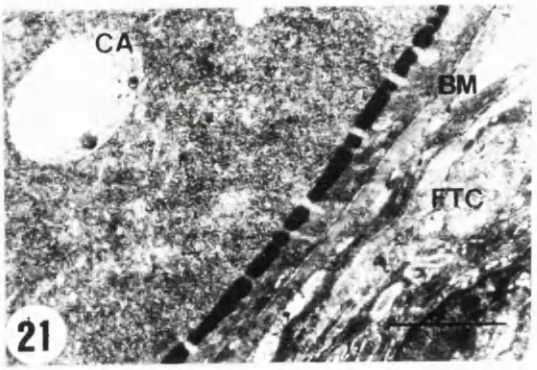

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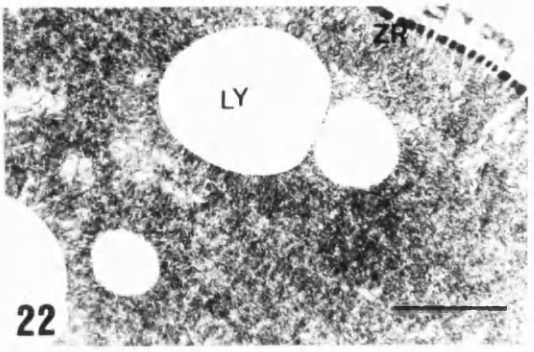

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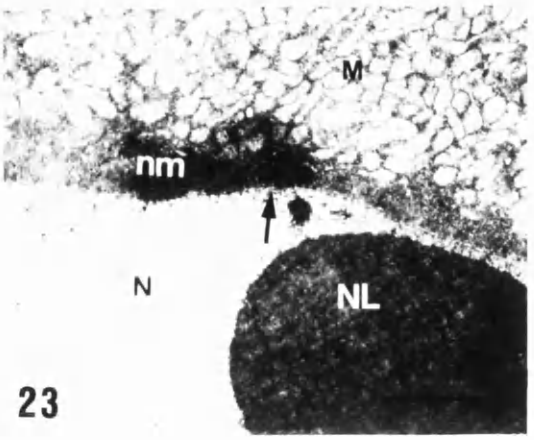


23

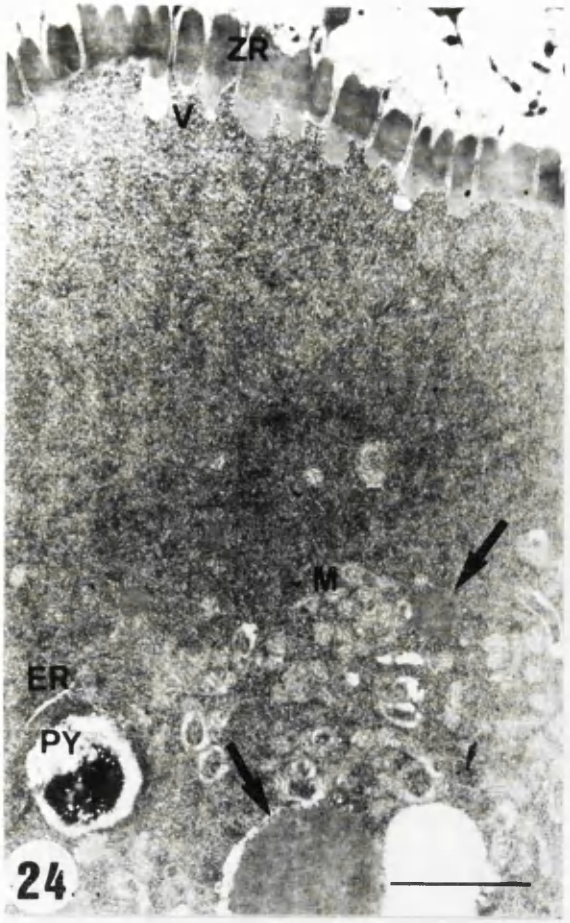
Figure 24. Electron micrograph of vitellogenic stage 4 oocyte showing the zona radiata is bipartite (ZR). Vesicles (V) are forming at the base of the zona radiata. Note the presence of endogenously formed protein yolk granules (arrow) and vesicles carrying protein yolk granules (PY) in the mid cortex of the oocyte. Mitochondria (M) and endoplasmic reticulum (ER) are also present.
Scale bar 1 μm.

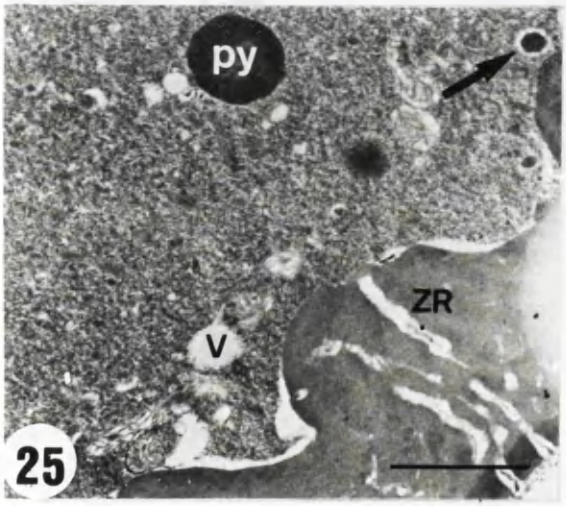
Figure 25. Electron micrograph showing the vesicles (V) carrying protein yolk granules (arrow). Protein yolk granules fuse to form bigger granules (PY). Note the accumulation of an electron-opaque material at the base of the zona radiata.
Scale bar 1 μm.

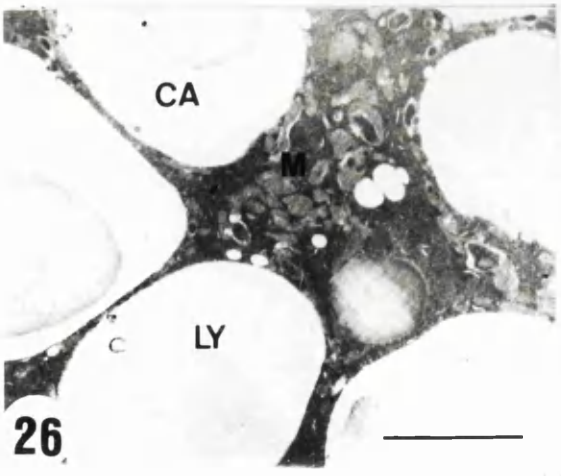
Figure 26. Electron micrograph of the oocyte inner cortex showing the presence of lipid yolk droplets (LY) of different sizes. Cortical alveoli (CA) are also present. The remaining cytoplasm contains mitochondria (M).
Scale bar 2 μm.

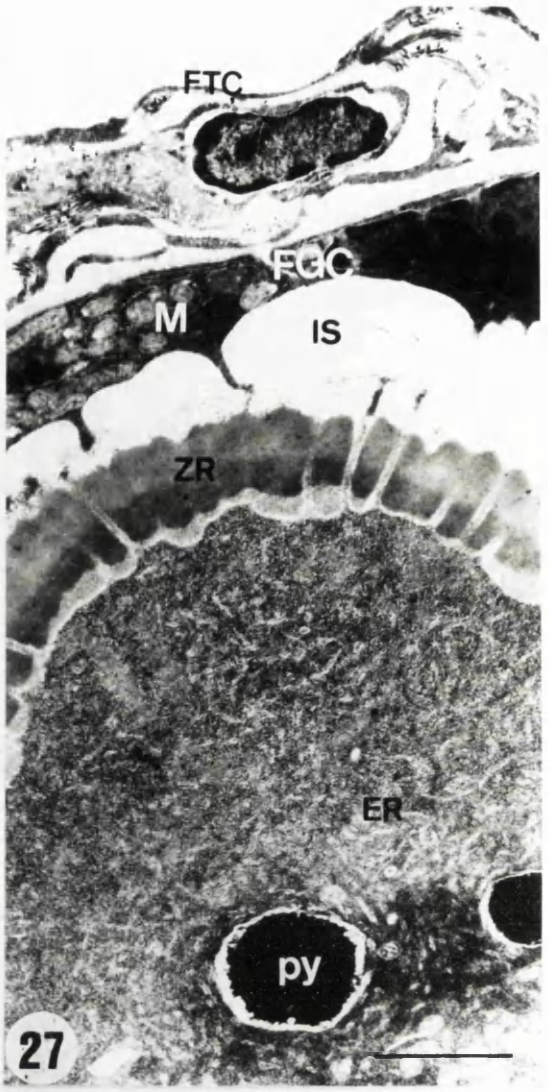
Figure 27. Electron micrograph showing the follicle theca cells (FTC) and follicle granulosa cells (FGC). Follicle granulosa cells contain mitochondria (M) and cytoplasmic processes. The intercellular space (IS) is electron-opaque, and a similar substance appears at the base of the zona radiata (ZR). The periphery of the oocyte contains small stalks of rough endoplasmic reticulum (ER) and vesicles carrying protein yolk granules (PY).
Scale bar 1 μm.

Figure 28. Electron micrograph of vitellogenic stage 4 oocyte showing the follicle cells (FC) containing branches of rough endoplasmic reticulum (RER). The intercellular space (IS) is electron-opaque. Vesicles (V) form at the base of the zona radiata and pinch off into the periphery of the oocyte (OO).
Scale bar 1 μm.


ZR
V
M
ER
PY
24


py
ZR
V
25


CA
M
LY
26


FTC
FGC
M
IS
ZR
ER
py
27

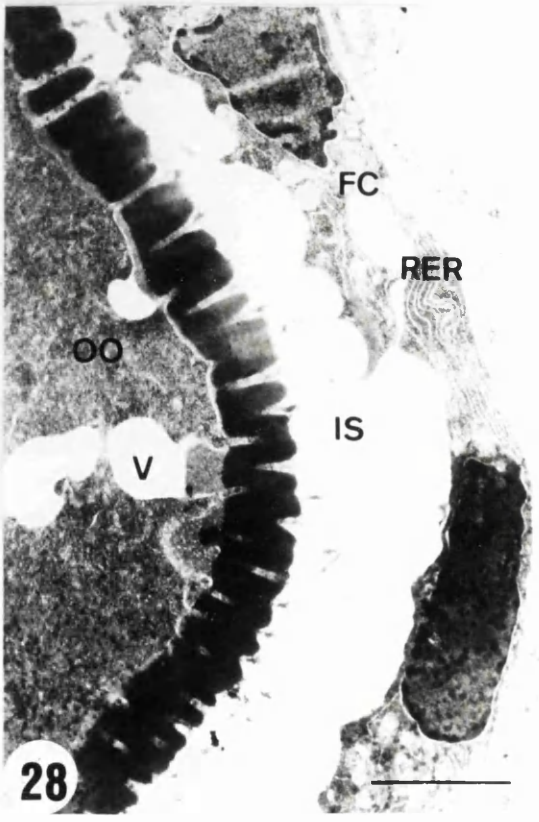
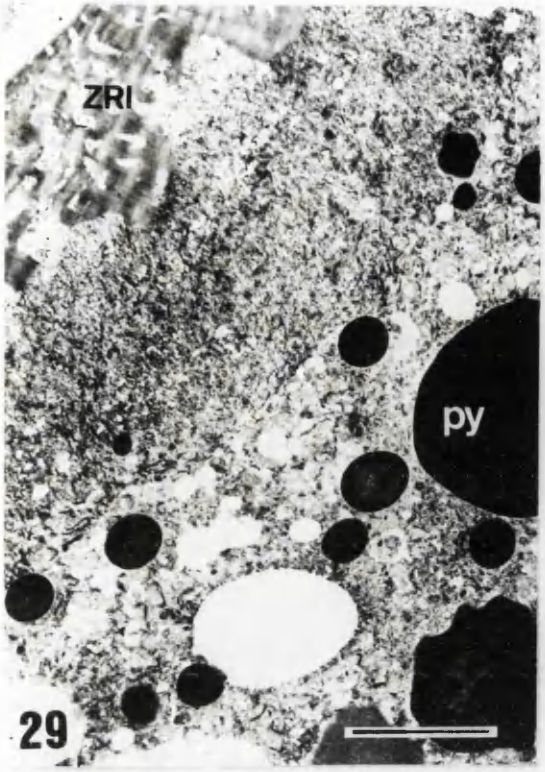

FC
RER
OO
IS
V
28

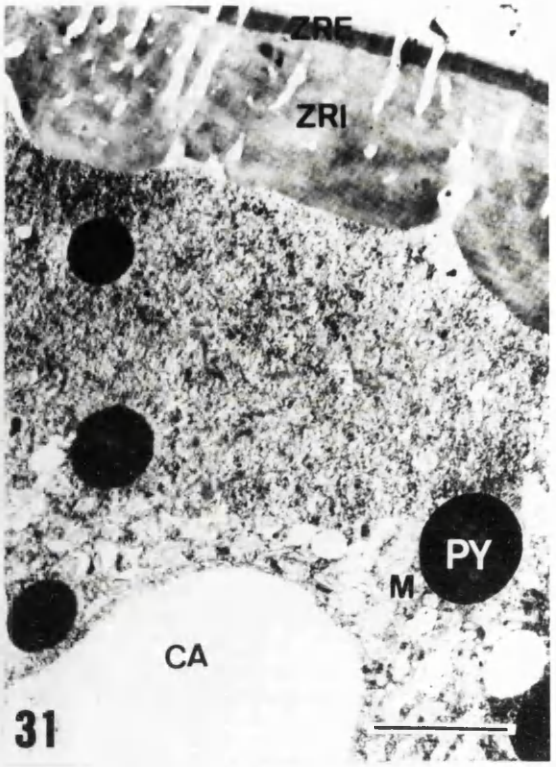
Figure 29. Electron micrograph of late vitellogenic stage 4 oocyte showing the formation of zona radiata interna (ZRI) adjacent to the oolemma. The periphery of the oocyte is dense. Protein yolk granules (PY) are increased in number, some are membrane-bound and the others are non-membranous. Smaller protein yolk granules fuse to become bigger and occupy outer cortex.
Scale bar 2 μm.

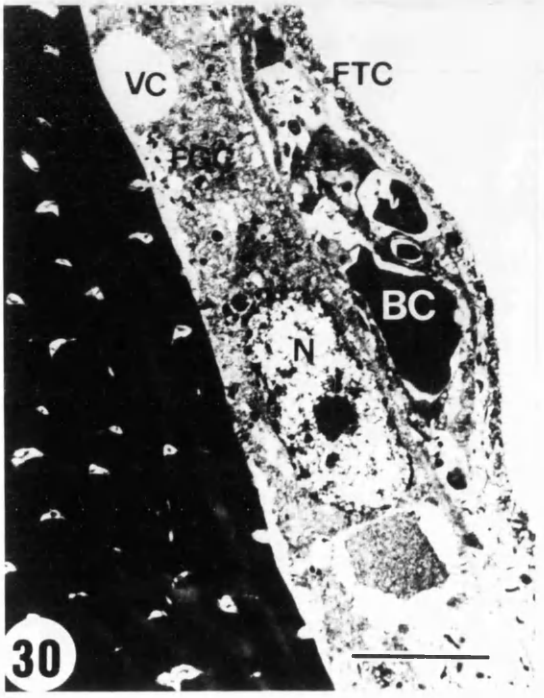
Figure 30. Electron micrograph of follicle theca cells (FTC) and follicle granulosa cells (FGS). Note the blood capillaries (BC) in the layer of follicle theca cells. Follicle granulosa cells containing nucleus (N) with a single nucleolus, mitochondria and vacuoles (VC) in the cytoplasm. The intercellular space is occluded.
Scale bar 2 μm.

Figure 31. Electron micrograph of the oocyte showing the tripartite zona radiata, the zona radiata externa (ZRE) and zona radiata interna (ZRI). The peripheral cytoplasm is dense containing protein yolk granules (PY). Cortical alveoli (CA) are present in the outer cortex. The outer cortex also contains mitochondria (M) and small protein yolk granules (PY).
Scale bar 2 μm.

Figure 32. Electron micrograph of the oocyte periphery. The zona radiata (ZR) become thick and organised. Note the presence of cortical alveoli (CA) in the peripheral cytoplasm of the oocyte.
Scale bar 2 μm.


ZRI
py
29


ZRE
ZRI
PY
M
CA
31


VC
FTC
BC
N
30

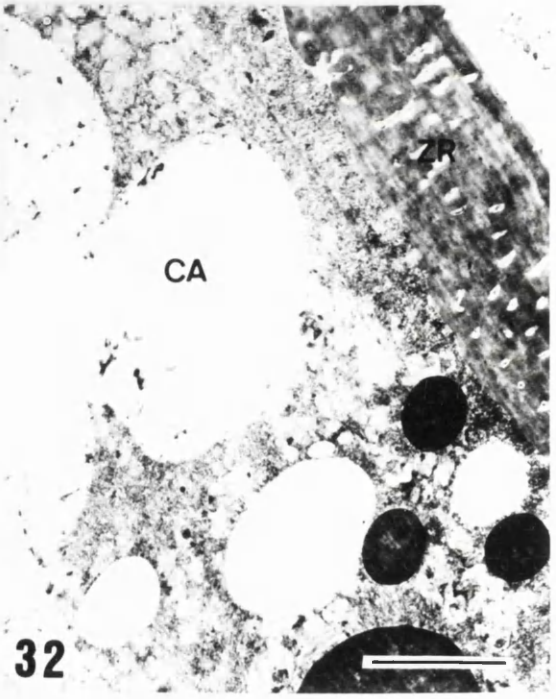

ZR
CA
32

Figure 33. Electron micrograph of follicle wall of the post-vitellogenic oocyte. Follicle granulosa cells have greatly enlarged and contain an electron-opaque vacuole (VC) and dense bodies (DB) and mitochondria (M). Nucleus (N) contains dispersed chromatin. Scale bar 2 μm.

Figure 34. Electron micrograph showing the zona radiata externa (ZRE) and zona radiata interna (ZRI). Note the space formed between the zona radiata and oolemma by microvilli being pulled back. Vesicles (V) filled with protein yolk granules are still present in the periphery. The outer and mid cortex are occupied by protein yolk granules (PY), cortical alveoli (CA) and mitochondria (M).
Scale bar 2 μm.

Figure 35. Electron micrograph showing the zona radiata (ZR). Note the presence of dense fibriller cortical alveoli (CA) beneath the zona radiata in the periphery of the oocyte. Protein yolk granules are membrane-bound and maintain structural integrity. The outer and mid cortex is occupied by cortical alveoli, protein y granules and mitochondria (M).
Scale bar 2 μm.

Figure 36. Electron micrograph showing the presence of less dense and granular cortical alveoli (CA) in the periphery of the oocyte beneath the zona radiata (ZR).
Scale bar 2 μm.

Figure 37 & 38. Electron micrograph of the mid cortex of the oocyte showing the dense cytoplasm which contains ribosomes, mitochondria (M) and protein yolk granules (PY). A few small lipid yolk droplets (LY) are also present. Small protein yolk granules coalesce and become bigger.

Scale bar 5, 1 μm.

Figure 39, 42 & 43. Electron micrograph of inner cortex of the oocyte showing the presence of lipid yolk (LY), a few cortical alveoli (CA) and small protein yolk granules (PY). Protein yolk granules differ in electron density. The less electron-dense protein yolk granules are mostly surrounded by lipid yolk droplets. Mitochondria (M) are present in the spaces between protein yolk and lipid yolk.

Scale bar 1, 2, 1 μm.

Figure 40. Electron micrograph of the peripheral cytoplasm of the oocyte showing the presence of vesicles (V) filled with protein yolk granules (PY).

Scale bar 2 μm.

Figure 41. Electron micrograph of the mid cortex of the oocyte showing the presence of endoplasmic reticulum (ER) and large protein yolk granules (PY).

Scale bar 1 μm.

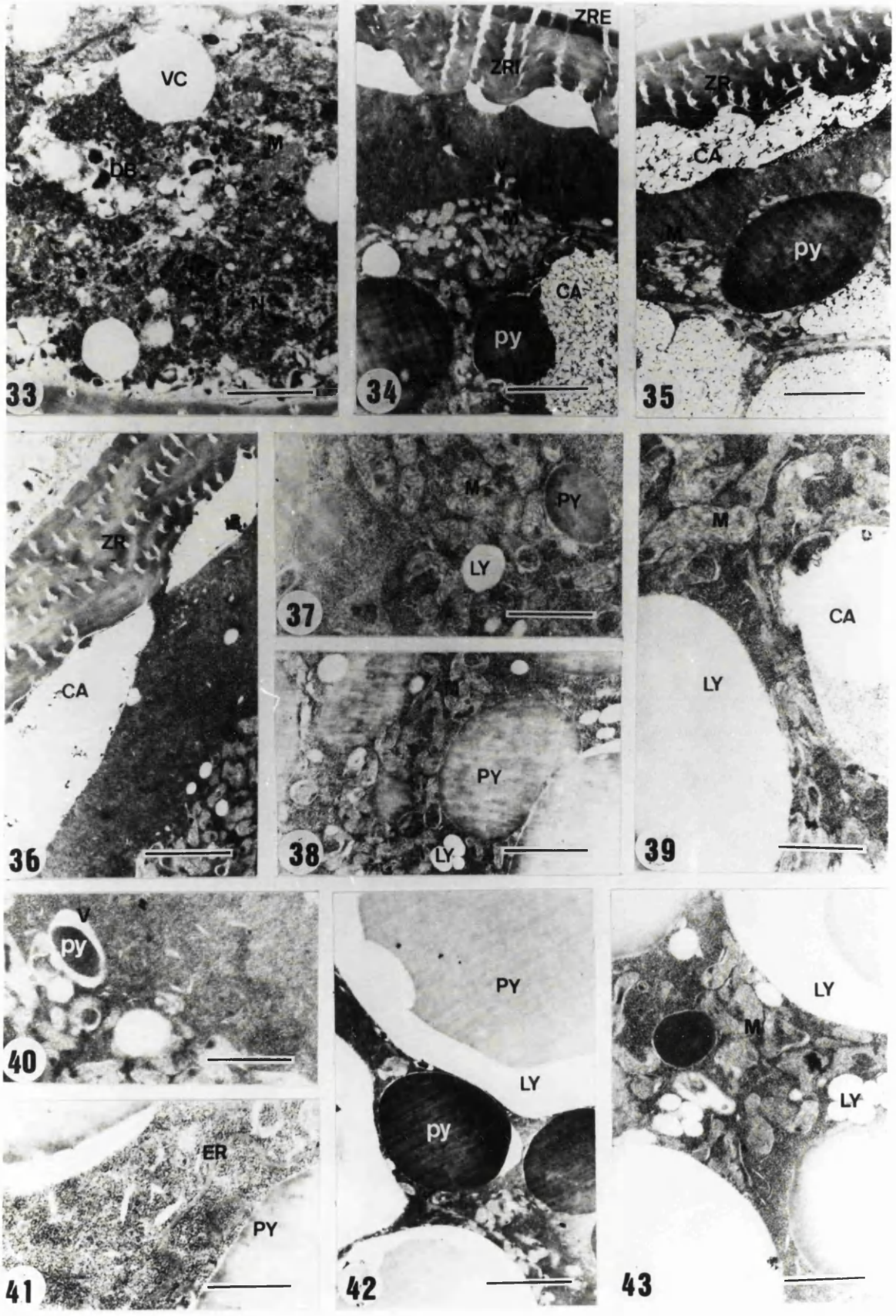

VC
M
DB
N
33
ZRE
ZRI
V
M
CA
py
34
ZR
CA
M
py
35
ZR
CA
36
M
PY
LY
37
PY
LY
38
M
CA
LY
39
V
py
40
PY
LY
py
42
LY
M
LY
43
ER
PY
41

Figure 44. Electron micrograph of follicle wall of maturation stage oocyte. Note the abundance of vacuoles (VC), and presence of dense bodies (DB) in the follicle granulosa cells (FGC). The nucleus (N) contains dispersed chromatin. Cytoplasm contains distorted mitochondria (M) and endoplasmic reticulum (ER).
Scale bar 2 μm.

Figure 45. Electron micrograph of a maturation stage oocyte showing the perivitelline space between the zona radiata (ZR) and oocyte (OO). Endoplasmic reticulum (ER), distorted mitochondria (M) and protein yolk granules (PY) are present in the peripheral cytoplasm.
Scale bar 2 μm.

Figure 46. Electron micrograph of outer and mid cortex of the oocyte showing protein yolk granules (PY) which have become bigger by fusion of small protein yolk granules. The cytoplasm between these protein yolk granules is occupied by mitochondria (M).
Scale bar 2 μm.

Figure 47. Electron micrograph showing solid zona radiata (ZR). The peripheral cytoplasm is occupied by a layer of cortical alveoli (CA). Few protein yolk granules (PY) are present. Mitochondria (M) are present in between the spaces of cortical alveoli and protein yolk granules.
Scale bar 2 μm.

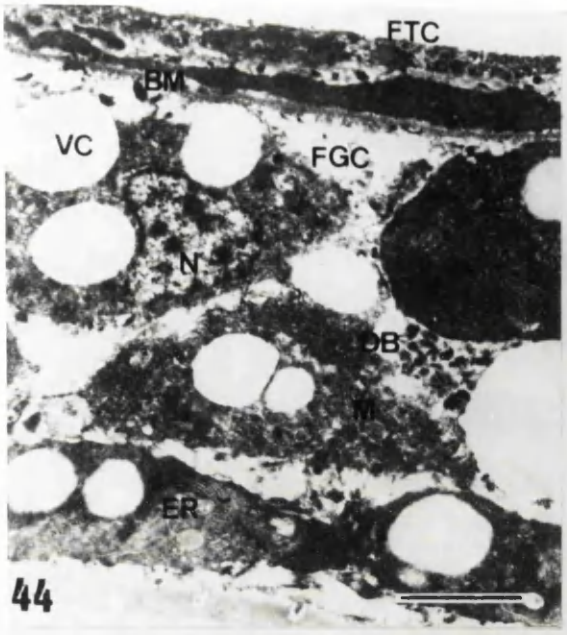
Figure 48 & 49.

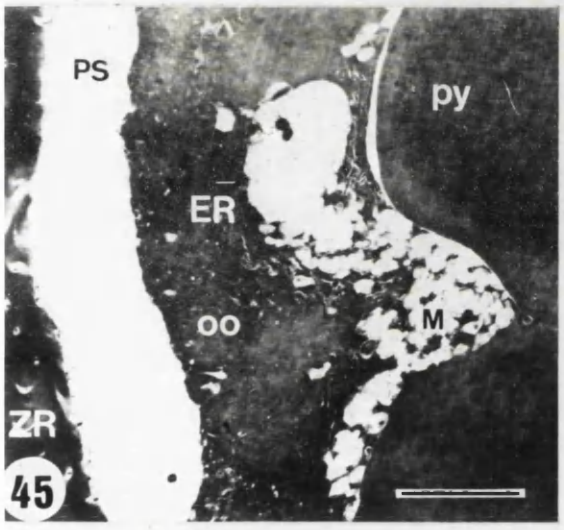
Electron micrograph showing release of contents of the cortical alveoli (CA)(cortical reaction) into the perivitelline space (PS) beneath the zona radiata (ZR).

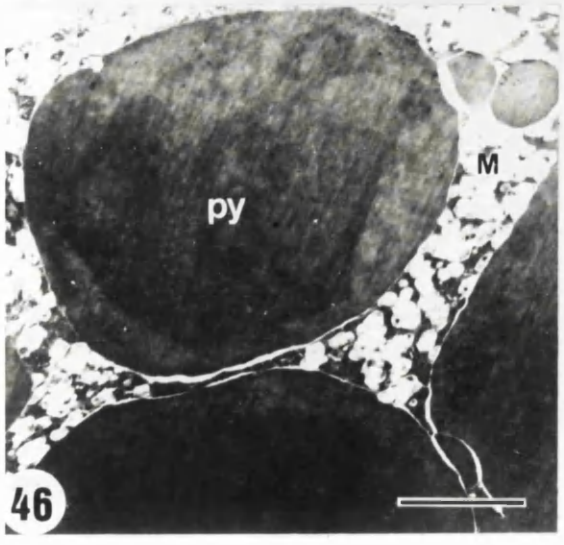
Scale bar 2, 1 μm.

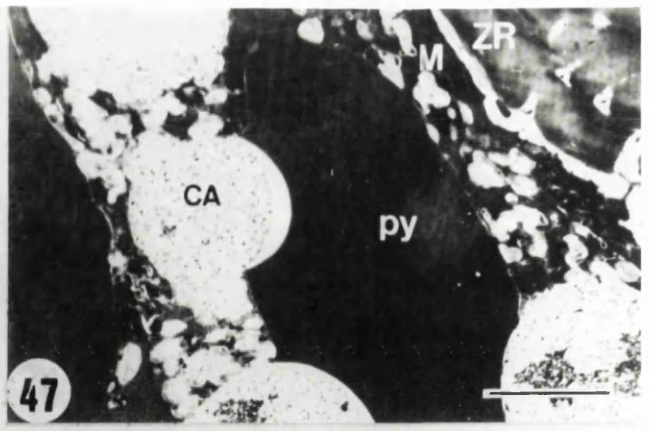
Figure 50. Electron micrograph of the oocyte showing the presence of cortical alveoli (CA) throughout much of the peripheral cytoplasm.

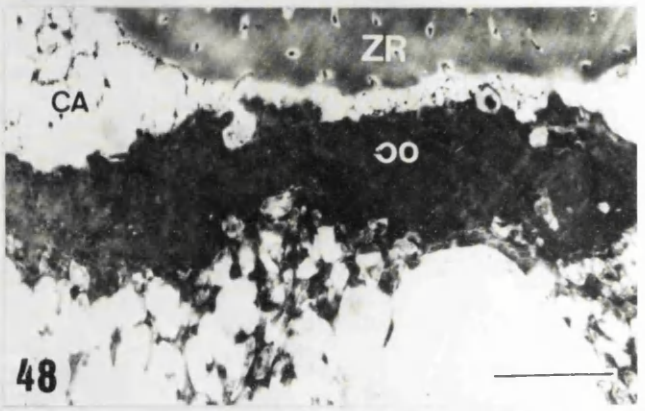
Scale bar 2 μm.

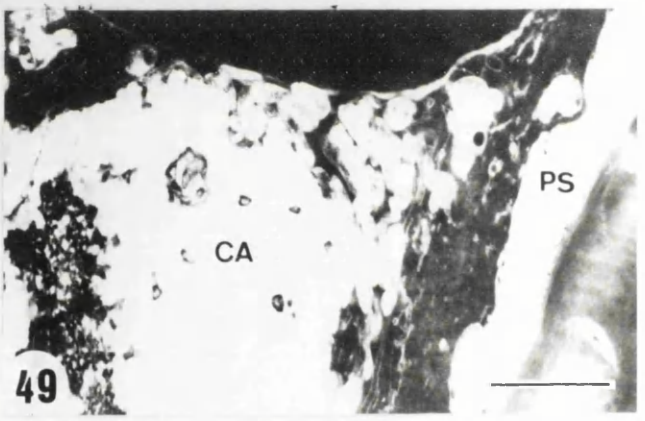

FTC
BM
VC
FGC
N
OB
M
ER
44

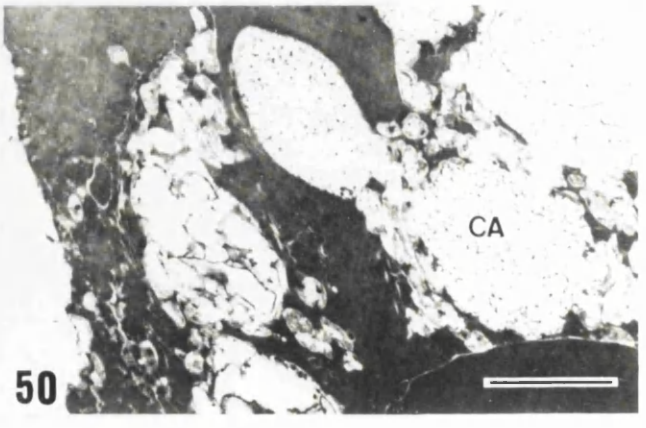

PS
py
ER
oo
M
ZR
45


py
M
46


M
ZR
CA
py
47


ZR
CA
oo
48


PS
CA
49


CA
50

Chapter 5

The effects of copper on post fertilization eggs and larvae of *C. harengus*

INTRODUCTION

Copper is biologically essential in trace amounts in respiratory enzymes of fish but in larger amounts causes adverse effects (Bryan, 1971). A number of sublethal and lethal responses, arising from exposure to copper have been reported in adult fish (Baker, 1969; Eisler and Gardner, 1973; Segner, 1987). Shazili and Pascoe (1986) and Westernhagen *et al.* (1979) examined the effects of heavy metals, *viz.* cadmium, copper, zinc and lead, on eggs of rainbow trout, *Salmo gairdneri*, and herring *Clupea harengus*. They observed an uptake of the heavy metals by eggs and showed that copper was highly toxic compared to cadmium, zinc and lead. Eggs of king salmon, *Oncorhynchus tshawytscha*, hatched normally in 0.08 ppm copper although fry died when exposed to 0.04 ppm copper (Hazel and Meith, 1970). Blaxter (1977) reported that copper reduced the percentage of successful fertilizations and the length of the incubation period and caused a high level of mortality in the larvae of the herring *C. harengus*. Similar results were observed when eggs and larvae of trout, *Salvelinus fontinalis*, were exposed to copper (McKim and Benoit, 1971). The effects of zinc, aluminium and mercury on the post fertilization eggs and larvae of *C. harengus* from Milford Haven and Swansea Bay, South Wales, U.K. were determined. There was reduction in egg volume and deformation of the jaws, head, optic capsule, otic capsule and vertebral column of the larvae (Somasundaram *et al.*, 1984; Mohammad-Nagib, 1987; Jastania, 1989). Alderdice *et al.* (1979b) observed changes in egg volume, yolk volume, and volume of the perivitelline space in eggs of the Pacific herring, *Clupea pallasi* during incubation in a variety of salinities and cadmium concentrations. McKim (1977) conducted a series of toxicity experiments on

all stages of fish from eggs to adults in cadmium, copper, chromium, lead and zinc and reported that the embryo-larval and early juvenile stages are generally more susceptible to the heavy metals than older fish. In other studies it was also described that eggs of fish are more resistant than hatched fry or alevins to cadmium and zinc (Skidmore, 1965; Eaton *et al.*, 1978; Rombough and Garside, 1982). Mount (1968) and Mount and Stephan (1969) studied the effects of long-term exposure of fish to copper. They exposed ***Pimephales promelas*** in copper through a complete life cycle and showed that spawning was reduced, the fish were smaller and sexual development was inhibited.

Other pollutants have been tested on fish eggs and larvae, *viz.* sulphuric acid pollutants on herring by Kinne and Rosenthal (1967), dinitrophenol on herring by Rosenthal and Stelzer (1970), oil dispersants on herring by Wilson (1974, 1976), cadmium on herring by Rosenthal and Sperling (1974) and by Westernhagen *et al.* (1974) and cadmium on flounder by Westernhagen and Dethlefsen (1975). In these studies, feeding behaviour, activity, heart rate, as well as hatching success and abnormal development, were used to assess the effects of the pollutants.

Abdullah and Royle (1972) and Humphrey *et al.* (1979) reported high concentration of copper, about 3 ppm in water, in the Bristol Channel, South Wales. The present study was conducted to examine the effects of various concentrations of copper on egg volume, volume of the perivitelline space, yolk volume and to look for possible deformities in larvae of *C. harengus*. These effects have been recorded when this species has been exposed to zinc in a similar manner to the present work (Somasundaram *et al.*, 1984).

Materials and Methods

Adults of *C. harengus* were collected from Milford Haven, South Wales, U.K. during March 1989 (Fig. A). Eggs were stripped from a single female and artificially fertilized (Alderdice, 1979) using sperm obtained from several males. Immediately after fertilization, samples of fertilized eggs were placed either in clean artificial seawater, or with a salinity of $20^{o}/oo$ (the mean salinity over the spawning grounds in Milford Haven) or in test solutions which contained either 0.01, 0.03 or 0.05 ppm copper prepared from a stock solution of $CuSO_4.5H_2O$ in artificial seawater with a salinity of $20^{o}/oo$. Both control and test solutions were continuously aerated at $8\pm1^{o}C$ (ambient temperature) and renewed every two days in order to maintain a reasonably constant concentration of copper in the test solution by countering any possible adsorption by the glassware or uptake by the eggs.

During the incubation period, measurements of egg volumes were made using an occular micrometer and the total volume obtained from samples of 15-20 eggs during each daily observation. Since the eggs are ellipsoidal, the volume was calculated from a measurement of the minor (d_1) and major (d_2) diameter, using the following formula:

$$V = \frac{4}{3\pi} \frac{(d_1 + d_2)^3}{4}$$

Similar measurements were made for yolk volume and the volume of the perivitelline space by subtracting the yolk volume from the total egg volume.

At hatching, the frequencies of any mouth and vertebral column abnormalities were determined from random samples of 20-30 larvae. The incubation period (time from fertilization to 50% hatching) was determined in two experimental tests. Body length and eye and otic capsule diameters of a random sample of 20-30 larvae at hatching were also measured. P (probability) was obtained by using student's t-test.

1. The effects of copper on post-fertilization *C. harengus* eggs:

Observations:

The total egg, perivitelline space and yolk volumes, measured in the present study, are shown in Tables 1 and 2 and Figures 1 to 4. Alderdice *et al.* (1979b) suggested a two stage developmental process. The first stage, which occurs 40-80 hrs after fertilization, involves volume adjustment by passive osmoregulation which includes equilibrium adjustments in the perivitelline fluid, swelling of the egg to its maximum volume, a decrease in the volume and a subsequent reduction in the total egg volume. The second stage, which occurs 80-100 hr after fertilization, involves volume adjustment by active osmoregulation in which there is an increase in the egg volume, perivitelline space and yolk volume. Later the yolk volume begins to diminish and the perivitelline space increases followed by a decline in the egg volume which continues until hatching.

During the present study a passive volume adjustment took place at 44-84 h after fertilization followed by active osmoregulation at 124-144 hr, during which the volume of the eggs, perivitelline spaces and yolk volumes were gradually reduced until hatching (Fig. 1).

Eggs incubated in 0.01 ppm copper showed no significant change in egg volume, perivitelline space or yolk volume compared to those in the controls. Passive and active osmoregulation took place over a similar period to that in the controls (Fig. 2).

Eggs incubated in 0.03 ppm copper showed a significant reduction in egg volume compared with that of the controls ($P<0.05$) during the first 44 hr but then the volume of the egg and perivitelline space remained similar to those of the controls during the period of active osmoregulation. There was a significant decrease in both egg volume and perivitelline space at 184 hr after fertilization ($P<0.05$) which continued until hatching. The yolk volume was similar to that in the controls (Fig. 3).

Eggs incubated in 0.05 ppm copper were similar in volume to the controls (1.69-1.91 mm^3) at 24 hr after fertilization, but at 44 hr they swelled ($P<0.05$) and then their volume was reduced continuously. A slight increase in volume was observed during active osmoregulation but was less than in the controls ($P<0.01$). At hatching the mean egg volume was 1.55 ± 0.07 mm^3 while in the controls the egg volume was 1.85 ± 0.03 mm^3 (Table 1, Figure 4). The perivitelline space was enlarged during passive osmoregulation but later became smaller until hatching. The yolk volume decreased at 24 hr, then started increasing until 44 hr, and after 84 hr remained unchanged until hatching (Fig. 4). A number of the eggs incubated in 0.03 and 0.05 ppm either underwent irregular cleavage, loss of blastomeres and decay of the embryos or development proceeded but embryos were unable to hatch.

Hatching:-

The incubation time was noted during two experimental tests. The larvae hatched after 16 to 17 days in the controls (Table 3). Similar results were obtained in specimens from eggs exposed to 0.01 ppm copper, but in those exposed to 0.03 and 0.05 ppm copper there was premature hatching after 15 days which continued until 18 days. Many embryos died in the egg stage, mostly at hatching. A number of malformed embryos were unable to hatch successfully and were stuck to the egg sac membrane.

DISCUSSION

In the eggs of teleosts, formation of the perivitelline fluid is attended by an expansion of the outer capsule, under increasing internal turgor pressure (Solemdal, 1967; Holliday and Jones, 1967; and Eddy, 1974). Perivitelline fluid consists of 60% protein, 35% lipid and 4% carbohydrates. The perivitelline colloids are in the form of glycoproteins or lipoproteins (Eddy, 1974). The chorion of teleost eggs is freely permeable to water so that changes in salinity of the external medium are followed after 4-6 hr by changes in the perivitelline fluid (Holliday, 1965; Weisbart, 1968; and Eddy, 1974). Consequently, the embryonic tissues are in direct contact with a perivitelline fluid which is very similar in osmolarity to that of the medium in which the eggs are being incubated (Holliday, 1969). Somasundaram *et al.* (1984) reported that in eggs of *C. harengus*, some accumulated zinc was bound to the chorion but the greater part accumulated in the perivitelline space. They also found that the period of highest uptake of zinc was at the beginning of tissue initiation. Westernhagen *et al.* (1979) stated that in *C. harengus*, the uptake of copper by eggs and larvae increased with increased exposure time and that high ambient copper levels result in a high copper content of eggs. Saliba and Krzyz (1976) suggested that the way in which heavy metals can enter the developing eggs is by absorption through the permeable chorion. Rosenthal and Sperling (1974) reported that in *C. harengus*, cadmium uptake takes place at the surface of the egg capsule, forming protein or mucopolysaccharide complexes and causing a change in the physico-chemical properties of the chorion. This change in the physico-chemical properties stresses the developing embryos, resulting in a shortened incubation period, a lower percentage of viable hatch and smaller larvae in those which do hatch.

In the present study high concentrations of copper caused reductions in egg volume and volume of the perivitelline space. A reduction in egg volume was also reported in the Pacific herring, *C. pallasi* when it was exposed to cadmium (Alderdice *et al.*, 1979b). Somasundaram *et al.* (1984) observed that the egg volume of *C. harengus* was reduced during passive osmoregulation and increased during the period of active osmoregulation when exposed to zinc. Mohammad-Nagib (1987) reported a reduction in total egg volume and perivitelline space in eggs of *C. harengus* exposed to seawater acidified with sulphuric acid. The reduction in egg volume of *C. pallasi*, resulting from cadmium, alters buoyancy in pelagic eggs which would alter their normal location in the water column and alter the salinity of their environment in a stratified water column (Alderdice *et al.*, 1979a).

In the present study the yolk volume declined at first but then it remained constant after 84 hr in specimens exposed to high concentrations of copper (0.05 ppm). Alderdice *et al.* (1979b) stated that the decrease in yolk volume in *C. pallasi* is a result largely of water loss during the period of high permeability of the plasma membrane. Somasundaram *et al.* (1984) concluded that the rapid decrease in yolk volume (62-96 hr) in *C. harengus* is due to increased energy utilised from the yolk by the fast growing blastoderm. McKim and Benoit (1971) reported that the utilization of yolk material was greatly retarded at 0.032 ppm copper in trout, *S. fontinalis*, alevins and four weeks longer were required for complete resorption of yolk compared with the time required for the process in the controls.

The present study found that in concentrations of 0.03 and 0.05 ppm copper, in affected embryos either mortality occurred at the blastomere stage or there was irregular development following the blastomere stage

which resulted in death of these embryos. A high mortality of herring eggs kept in 0.03 ppm copper during incubation is also reported by Steel *et al.* (1973) and Blaxter (1977). Somasundaram *et al.* (1984) reported that zinc uptake occurs in the eggs of *C. harengus* which accumulates in the embryo and yolk at a critical period (cleavage stage) in embryonic development. The resulting zinc-induced altered rate of development caused post-hatching larval deformities. Mori (1979) reported that the eggs of *Carassius auratus* were more sensitive to cadmium and mercury at the blastomere stage than at the eyed stage. However, the blastomere stage was found to be resistant to cadmium, copper and zinc than other stages of embryonic development and the stage most sensitive to cadmium and zinc was later, at ten days post-fertilization development in embryos of trout (Shazili and Pascoe, 1986). Rombough and Garside (1982) also observed peak mortality during gastrulation and axiation as well as during development of vitelline circulation and shortly before hatching when eggs of trout were exposed to cadmium.

In the present study the incubation time was reduced in eggs exposed to high concentrations of copper (0.03 or 0.05 ppm). McKim and Benoit (1971) reported premature hatching in trout, *S. fontinalis*, from eggs exposed to copper concentrations of 0.032 ppm and that twenty-five percent mortality occurred as the embryos emerged from the eggs. Steele *et al.* (1973) reported a low survival rate in herring eggs kept in copper solutions with concentrations above 0.01 ppm and hatching occurred 1-3 days earlier than in the controls. Blaxter (1977) recorded a much reduced survival rate in *C. harengus* eggs kept during the whole incubation period in a copper solution of 0.03 ppm and hatching tended to be earlier than in controls. Westerhagen *et al.* (1979) suggested that the hatching enzymes produced by the glands around the head region dissolve the chorion causing premature

hatching and death of the embryo. Somasundaram *et al.* (1984) reported that eggs of *C. harengus* exposed to 6 ppm zinc solutions ruptured easily when subjected to mechanical pressure. Premature hatching of *C. harengus* occurred in eggs kept in 1 ppm cadmium solutions (Rosenthal and Sperling, 1973). Eggs of *C. harengus*, when exposed to cadmium concentrations in excess of 1 ppm developed weakened capsules which proved to rupture (Rosenthal and Sperling, 1973; Westernhagen *et al.*, 1974; Westernhagen and Dethlefsen, 1975). Alderdice *et al.* (1979a) reported fragile Pacific herring eggs when exposed to cadmium and attributed it to reduced tensile strength, caused by alterations in the egg capsule by bound cadmium. This could be the reason for the premature hatching in eggs of *C. harengus*, previously incubated in 0.03 and 0.05 ppm copper concentrations.

2. The effects of copper on *C. harengus* larvae

Observations:

2.1. *Body length, eye and otic capsule diameters at hatching*:-

The mean body length of newly hatched control larvae and those reared in copper concentrations are shown in Table 4. The larvae reared in test solutions were shorter than those in controls, the reduction in body length being proportional to the increase in copper concentration.

The eye diameter to body length ratio of larvae in the controls and copper solutions are shown in Figure 5. There was no significant difference in eye diameter in larvae hatched in 0.01 ppm copper compared with those in the controls. The eye diameters of larvae reared in 0.03 or 0.05 ppm copper were smaller than those in controls.

The otic capsule diameter to body length ratios at hatching are presented in Figure 6. In higher concentrations of copper the size of the

otic capsules showed a significant ($P<0.01$) reduction compared with those in the controls.

2.2. *Deformities of larvae hatched in copper solutions*:-

A number of vertebral column abnormalities occurred in specimens reared in the test solutions (Figs. 7 to 16). Several types of deformities were observed:

(i) a curvature downward or upward of the vertebral column originating posterior to the yolk sac or above the anus (bent);

(ii) a combination of mid-body and tail-end curvature;

(iii) coiling of the vertebral column (curl) which may be divided into primary, secondary and tertiary degrees of twisting depending on the severity of coiling;

The severity of curvature generally increased with increased concentrations of copper. The incidence of deformities were correlated with copper concentrations. Most of the larvae hatched in 0.05 ppm copper concentrations showed pronounced curvature of the vertebral column and remained on the bottom of the culture jar, occasionally showing some wriggling movements. All deformities of the vertebral column were accompanied by serrated primordial fins.

The fatal abnormalities other than those of the vertebral column were: (a) jaw deformations resulting mainly in rudimentary lower jaws, i.e., 'sucker mouthed' condition; (b) reduction of the frontal skull and upper jaw bones, 'dogs head' or 'pugheadedness' (Hickey *et al.*, 1977); and, (c) protrusion of the eye ball 'exophthalmus condition' which only occurred in the larvae hatched in higher concentrations, 0.05 ppm, of copper (Figs. 17 to 25).

DISCUSSION

The exposure of eggs to copper induced deformities in newly hatched larvae of *C. harengus*. The proportion of abnormal larvae was related to the copper concentrations in the water. The reduction in the size of the eyes and otic capsule occurred in prematurely hatched larvae. Similar incidences of reduction in eye and otic capsule size were also observed in the larvae of *C. harengus* hatched from eggs previously exposed to zinc (Somasundaram *et al.*, 1984). Weis and Weis (1977) suggested that defects of eyes and head produced by mercury reflects an interference with the inductive processes resulting in a deformation of the fore brain.

Rosenthal and Alderdice (1976) reported that deformities of eye, otic capsule, jaws and vertebrae result from environmental stress imposed during incubation. The reduction in the size of the eyes and diameter of the otic capsules or, in some cases, complete absence of the otic capsules in larvae of the Baltic herring, *C. harengus* were observed when eggs were incubated in cadmium solutions (Rosenthal and Sperling, 1973). It is well known that copper interferes with calcium metabolism (Reid and McDonald, 1988) and calcium is required in the development of the skeleton of fish larvae (Rosenthal and Sperling, 1973). Otic capsule deformities would affect the equilibrium of the fish and thus impair swimming activity, which would affect feeding and escape from predators.

In the present study copper retarded normal development of the embryo which produced larvae with shorter bodies. Copper reduced the growth of alevins and juveniles of brook trout (McKim and Benoit, 1971). Takeshimurai *et al.* (1981) reported that the growth of fingerlings of catfish was affected by increasing the copper level in their food. Other studies showed a reduction in growth and sexual development and an inhibition of spawning

when fish was exposed to copper (Mount, 1968; McKim and Benoit, 1971; and Eaton, 1973). The trout alevins were significantly shorter than those of controls when exposed to mercury, cadmium and lead (Christensen, 1975). Rombough and Garside (1982) suggested that reduced growth is caused by cadmium interference with the normal assimilation of yolk proteins and that this is the reason for the slow absorption of yolk. In the present study the constant yolk volume in embryos after 84 hr post fertilization development compared to controls indicates a delay in yolk utilization which may account for small larvae at hatching.

Vertebral column abnormalities in the larvae are a common response to various forms of environmental stress during ontogenic development in fish (Rosenthal and Alderdice, 1976; and Wilson, 1976). Abnormal vertebrae were formed in *C. harengus* from British waters (Ford and Bull, 1933). Dethlefsen *et al.* (1975) reported that cadmium caused vertebral column flexure in the larvae of *Belone belone* and that these larvae were unable to swim. In the present study the most common abnormality was the curled and bent larvae in higher copper concentrations (0.05 ppm). A similar condition was observed in killifish, *Fundulus heteroclitus* embryos exposed to mercury and lead (Weis and Weis, 1977). Vertebral anomalies were also reported in blue gill, *Lepomis macrochirus* larvae (Eaton, 1970), rainbow trout, *S. gairdneri* alevins (Beattie and Pascoe, 1978), minnows, *Phoxinus phoxinus* (Bengtsson, 1974) and herring *C. harengus* larvae (Somasundaram *et al.*, 1984) when exposed to cadmium and zinc. Christensen (1966) suggested that environmental factors, such as low O_2 concentration in the water during the early hatching period, produce abnormal body curvatures in newly hatched fry. Heavy metals, including copper, disrupt ionic regulation and are known to be metabolic antagonists to calcium which is an essential element for the

development of skeletal structures (Somasundaram *et al.*, 1984). Calcium influx is impaired and efflux stimulated in rainbow trout, *S. gairdneri* exposed to copper (Reid and McDonald, 1988) by affecting Ca, ATP-ase enzymes (Shephard and Simkiss, 1978). Reader *et al.* (1988, 1989) reported that mineral uptake and skeletal calcification are affected by copper resulting in impaired metabolism and development. Any environmental factor which can alter the metabolic rate and rate of development of fish eggs may also influence the number of vertebrae (Fowler, 1970; Garside, 1966). Fonds *et al.* (1973) observed that the increase in the rate of embryonic development of garfish, *Belone belone*, resulted in a decrease in vertebral number. Embryos of *C. harengus* showed accelerated development in zinc solutions (Somasundaram *et al.*, 1984) and premature hatching in copper (Blaxter, 1977).

In the present study a large number of the larvae hatched following incubation in test solutions of copper had jaw anomalies. Most of the larvae were without jaws or with rudimentary jaws. Somasundaram *et al.* (1984) reported jaw abnormalities in *C. harengus* larvae incubated in zinc and suggested that the rudimentary jaws may be related to the shortened incubation period. Barahona-Fernandes (1982) again reported similar deformities, i.e., the reduction in the lower jaw, and sucker mouth or pugheadedness conditions, occurring in fingerlings of the hatchery-reared European sea bass, *D. labrox* which resulted from environmental factors in the hatchery. Similar deformities have been observed in the larvae of the Pacific herring, *C. pallasi*, hatched at a low temperature and salinity (Alderdice and Velsen, 1971).

In the present study the affected larvae hatching from eggs incubated in the various copper solutions suffered deformities which would not enable

them to maintain their equilibrium during swimming. Defective mouths and skeletal parts would make feeding impossible and prevent growth. All of these conditions would result in the subsequent death of the larvae from starvation.

TABLE 1. THE MEAN TOTAL EGG VOLUME (mm^3) OF *CLUPEA HARENGUS* L. POST FERTILIZATION EGGS INCUBATED IN THE CONTROL AND DIFFERENT COPPER SOLUTIONS (0.01, 0.03 OR 0.05 ppm).

Incubation time (hrs)	Mean Total Egg Volume (mm^3)					
	Control	0.01		0.03		0.05	
	x	x	P	x	P	x	P
24	1.85	1.95	(>0.1)	1.75	(<0.05)	1.80	(>0.1)
44	2.05	2.05	(>0.1)	1.90	(<0.05)	2.15	(<0.05)
64	2.10	2.07	(>0.1)	1.95	(>0.1)	2.19	(<0.05)
84	2.00	2.10	(>0.1)	1.85	(<0.05)	1.65	(<0.01)
104	1.90	1.90	(>0.1)	1.78	(<0.05)	1.62	(<0.01)
124	1.85	1.90	(>0.1)	1.78	(<0.1)	1.62	(<0.01)
144	2.00	2.00	(>0.1)	1.90	(<0.05)	1.80	(<0.01)
184	1.91	1.90	(>0.1)	1.80	(<0.05)	1.70	(<0.01)
224	1.90	1.85	(>0.1)	1.85	(<0.05)	1.65	(<0.01)
244	1.98	2.00	(>0.1)	1.82	(<0.05)	1.60	(<0.02)
264	1.95	1.90	(>0.1)	1.86	(<0.05)	1.60	(<0.01)
340	1.85	1.88	(>0.1)	1.70	(<0.05)	1.55	(<0.01)

TABLE 2. THE MEAN PERIVITELLINE SPACE VOLUME (mm^3) OF *C. HARENGUS* L. POST FERTILIZATION EGGS INCUBATED IN THE CONTROL AND DIFFERENT COPPER SOLUTIONS (0.01, 0.03, 0.05 ppm).

Incubation time (hrs)	Mean Perivitelline space Volume (mm^3) Control x	0.01 x P	0.03 x P	0.05 x P
24	1.15	1.25 (>0.1)	1.13 (>0.1)	1.25 (<0.05)
44	1.36	1.38 (>0.1)	1.24 (<0.05)	1.50 (<0.05)
64	1.38	1.38 (>0.1)	1.30 (>0.1)	1.60 (<0.05)
84	1.44	1.42 (>0.1)	1.25 (<0.05)	1.20 (<0.05)
104	1.42	1.45 (>0.1)	1.28 (<0.05)	1.16 (<0.01)
124	1.40	1.41 (>0.1)	1.30 (>0.1)	1.20 (<0.05)
144	1.50	1.49 (>0.1)	1.32 (<0.05)	1.38 (<0.05)
184	1.52	1.48 (>0.1)	1.35 (<0.05)	1.18 (<0.01)
224	1.47	1.50 (>0.1)	1.28 (<0.05)	1.14 (<0.01)
244	1.52	1.51 (>0.1)	1.30 (<0.05)	1.09 (<0.001)
264	1.51	1.50 (>0.1)	1.41 (<0.05)	1.08 (<0.001)
340	1.49	1.52 (>0.1)	1.22 (<0.05)	1.01 (<0.001)

TABLE 3. INCUBATION PERIOD (TIME FROM FERTILIZATION TO 50% HATCHING) OF *C. HARENGUS* EGGS INCUBATED IN DIFFERENT COPPER SOLUTIONS.

Concentrations (ppm)	Incubation Period (Days) Test 1	Test 2	Mean
Control	17	16	16.5
0.01	16	16	16.0
0.03	16	15	15.5
0.05	15	15	15.0

TABLE 4. THE MEAN BODY LENGTH OF *C. HARENGUS* LARVAE AT HATCHING. EGGS INCUBATED IN DIFFERENT CONCENTRATIONS OF COPPER.

Concentrations (ppm)	Body length at hatching (mm) n	x	±S.D.
Control	25	7.92	± .62
0.01	20	7.50	± .70
0.03	20	5.81	± .67
0.05	25	4.23	± .41

n = Number of specimens.

Figure A. Map showing known spawning areas of *C. harengus* L. in the Milford Haven Estuary (After Clarke, 1984).

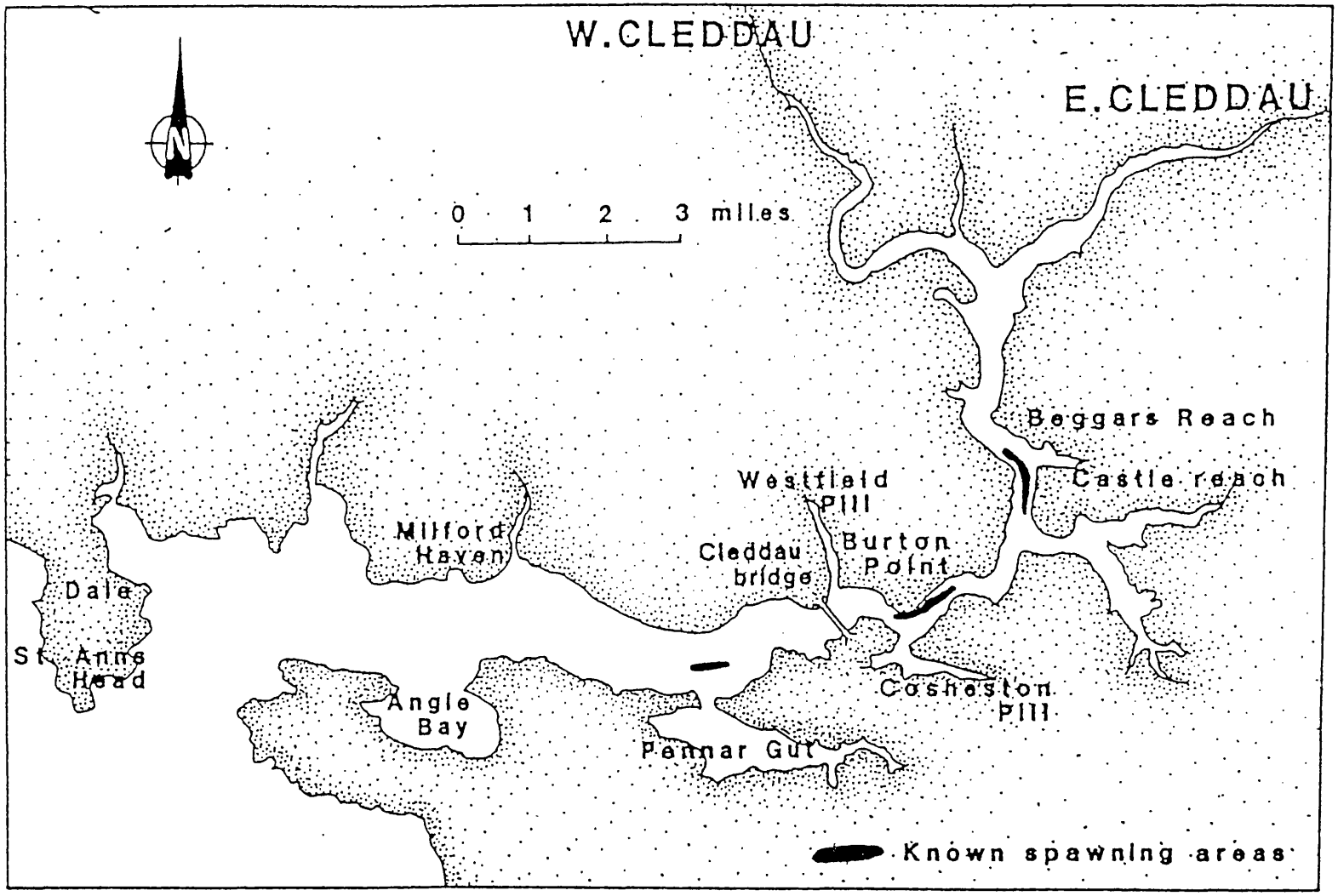

W.CLEDDAU
E.CLEDDAU
N
0 1 2 3 miles
Beggars Reach
Castle reach
Westfield Pill
Milford Haven
Cleddau bridge
Burton Point
Dale
St Anns Head
Angle Bay
Cosheston Pill
Pennar Gut
Known spawning areas

Figure 1. Egg volume of herring, *C. harengus* eggs (mm^3) incubated in artificial seawater (20^{o}/oo) at $7\pm1^{o}C$.

Figure 1.

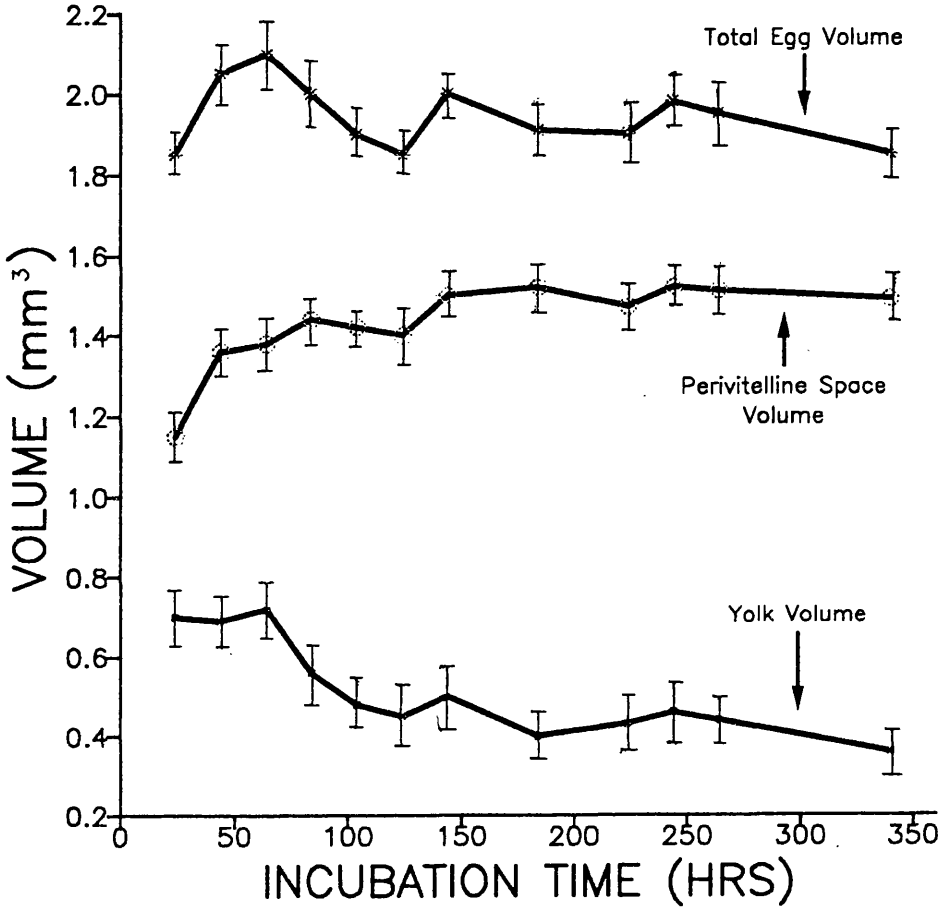

Total Egg Volume
Perivitelline Space Volume
Yolk Volume
VOLUME (mm³)
INCUBATION TIME (HRS)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
50
100
150
200
250
300
350

Figure 2. Egg volume of herring, *C. harengus* eggs (mm^3) incubated in 0.01 ppm copper solution ($20^{o}/oo$) at $7\pm1^{o}C$ compared with control.

------ 0.01 ppm copper

——— control.

Figure 2.

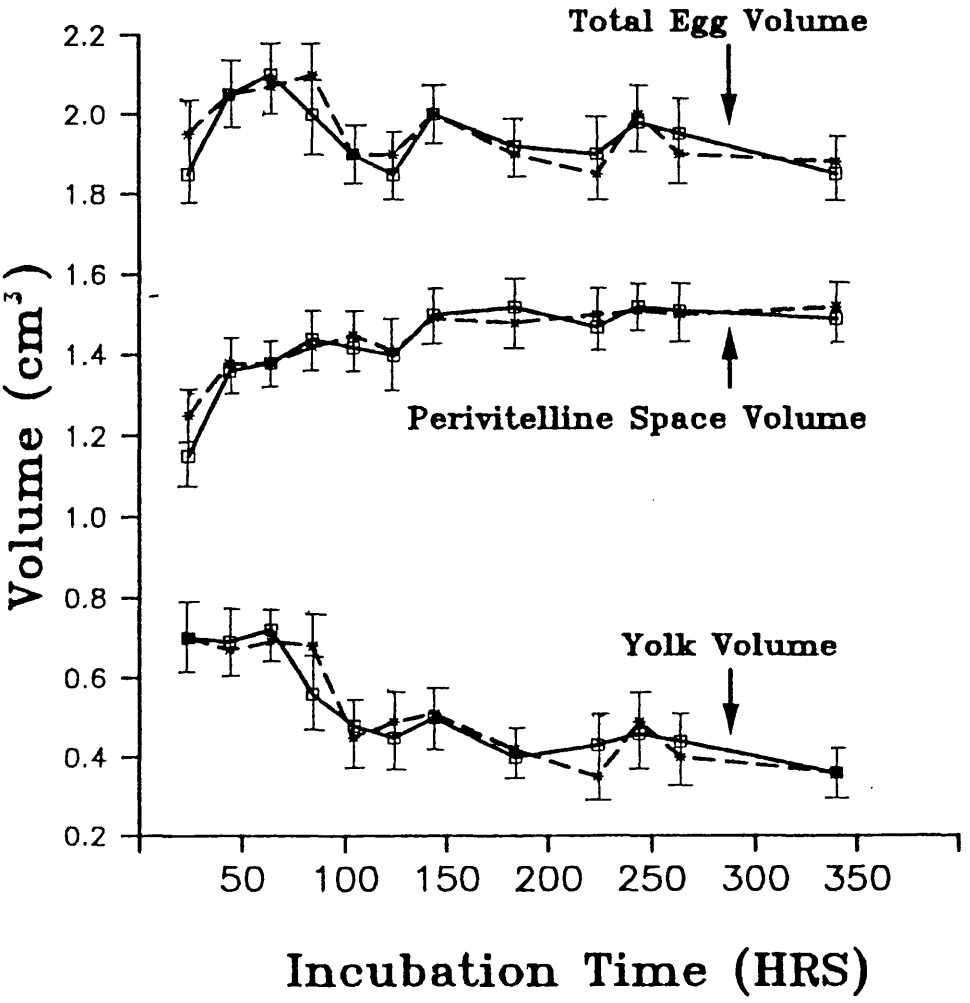

Total Egg Volume
Perivitelline Space Volume
Yolk Volume
Volume (cm³)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
50
100
150
200
250
300
350
Incubation Time (HRS)

Figure 3. Egg volume of herring, *C. harengus* eggs (mm^3) incubated in 0.03 ppm copper solution ($20^{o}/oo$) at $7\pm1^{o}C$ compared with control.

------ 0.03 ppm copper

——— control.

Figure 3.

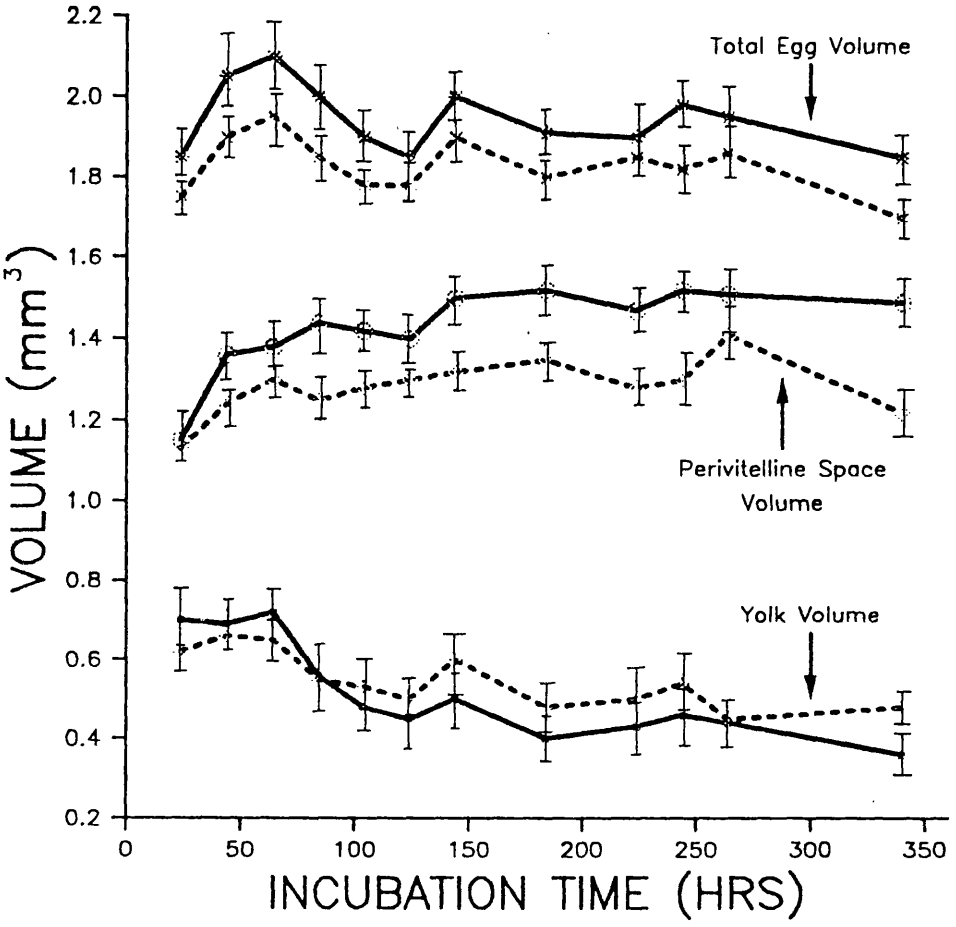

Total Egg Volume
Perivitelline Space Volume
Yolk Volume
VOLUME (mm³)
INCUBATION TIME (HRS)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
50
100
150
200
250
300
350

Figure 4. Egg volume of herring, *C. harengus* eggs (mm^3) incubated in 0.05 ppm copper solution ($20^{o}/oo$) at $7\pm1^{o}C$ compared with control.

------ 0.05 ppm copper

——— control.

Figure 4.

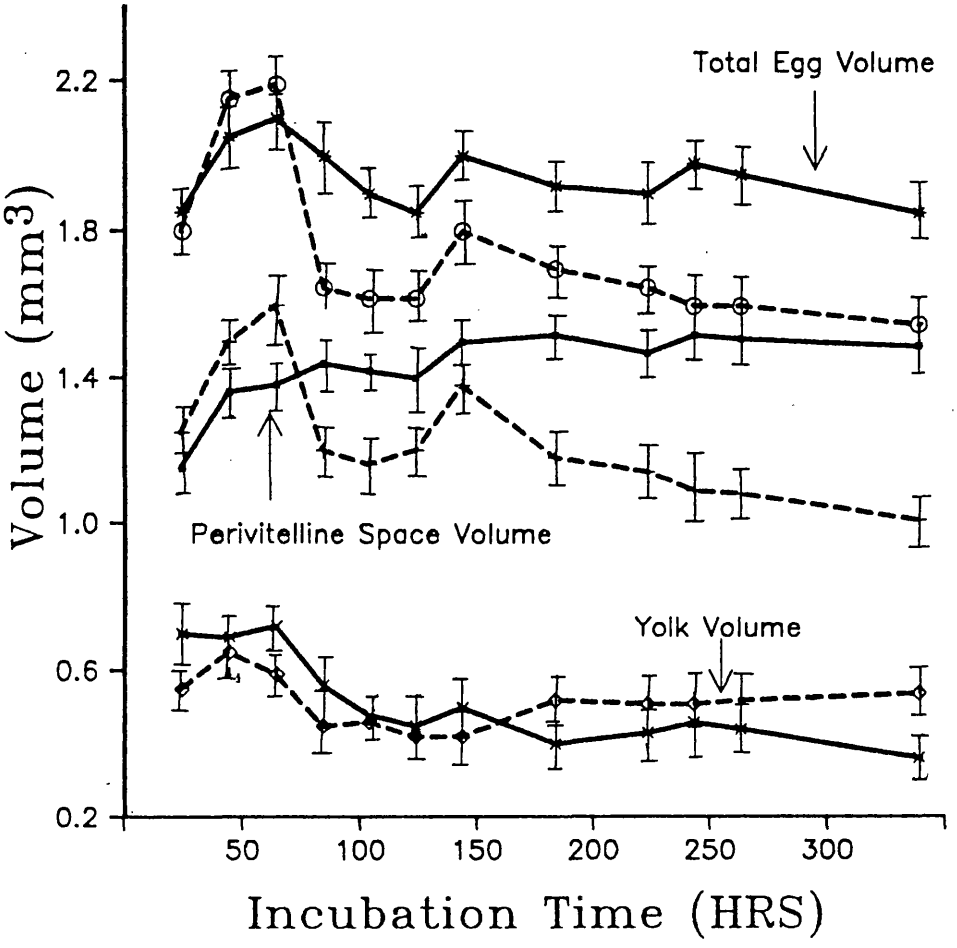

Total Egg Volume
Perivitelline Space Volume
Yolk Volume
Volume (mm3)
2.2
1.8
1.4
1.0
0.6
0.2
50
100
150
200
250
300
Incubation Time (HRS)

Figure 5. Histograms showing the ratio of eye diameter to body length of herring *C. harengus* larvae at hatching. Larvae hatched from eggs previously exposed to different copper concentrations during the incubation period. Significant difference from control (*** = $P<0.001$).

Figure 5.

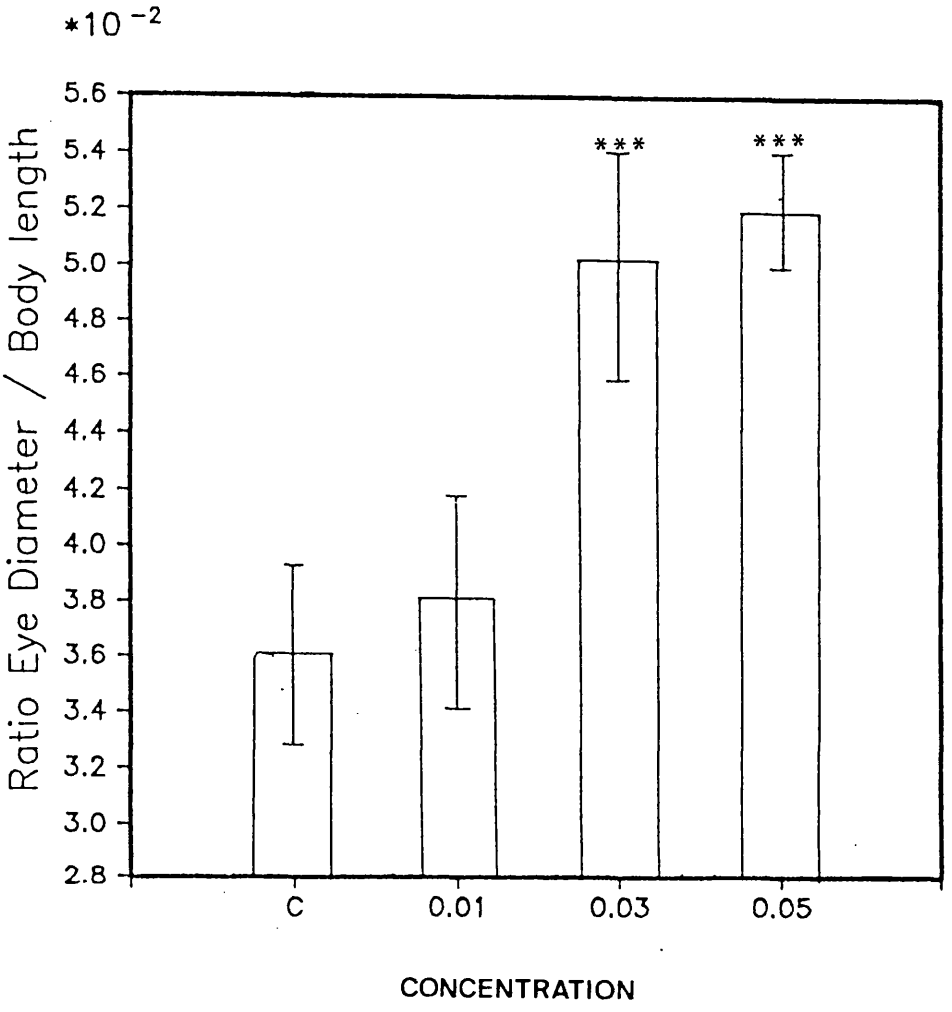
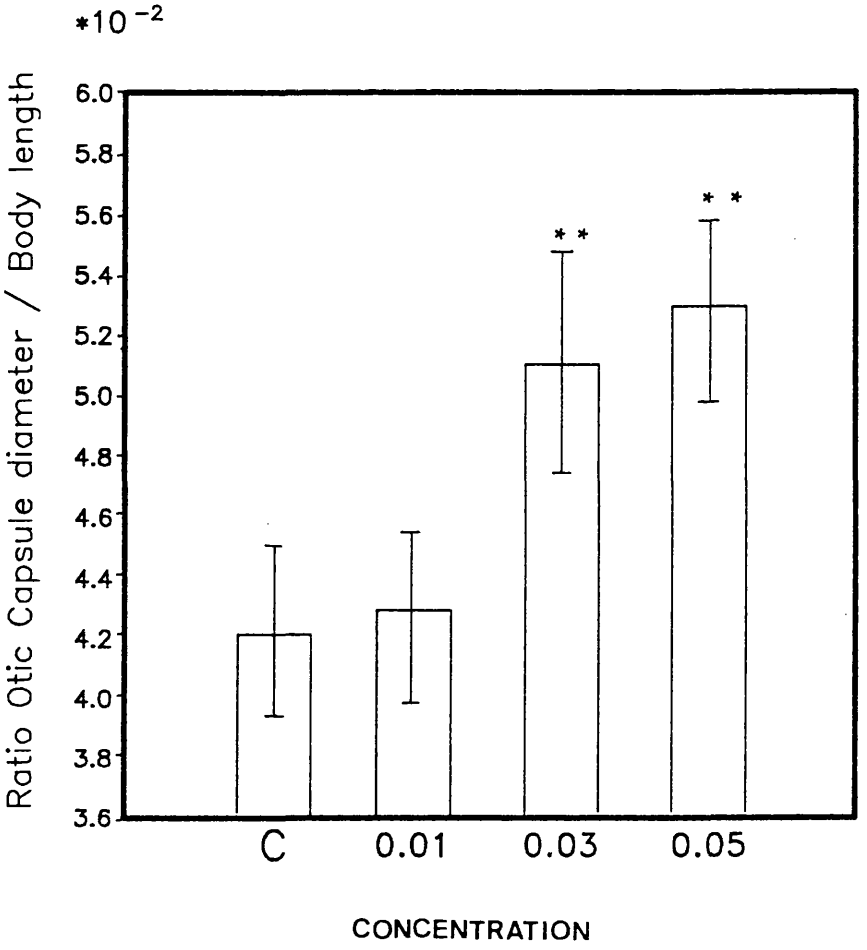

*10 -2
Ratio Eye Diameter / Body length
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
***
***
C
0.01
0.03
0.05
CONCENTRATION

Figure 6. Histogram showing the ratio of otic capsule diameter to body length of herring, *C. harengus* larvae at hatching. Larvae hatched from eggs previously exposed to different copper concentrations during the incubation period. Significant difference from the control (** = $P<0.01$).

Figure 6.


*10 -2
Ratio Otic Capsule diameter / Body length
6.0
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
* *
* *
C
0.01
0.03
0.05
CONCENTRATION

Photographs showing skeletal abnormalities of the newly hatched larvae of *C. harengus*.

Figure 7. Photograph showing a normal hatched larva.
Scale bar 0.2 mm.

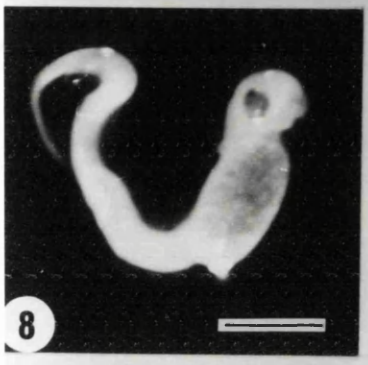
Figure 8/9. Photographs showing the vertebral column is twisted in a zig-zag shape.
Scale bar 0.2 mm.

Figure 10 & 11.
Photographs showing curvature of the vertebral column in the trunk region.
Scale bar 0.2 mm.

Figure 12, 13, 14 & 16.
Photographs showing the vertebral column of the larvae coiled in primary, secondary and tertiary degrees (knot-shape).
Scale bar 0.2 mm.

Figure 15. Photograph showing the vertebral column curvature posterior to the yolk sac.
Scale bar 0.2 mm.


7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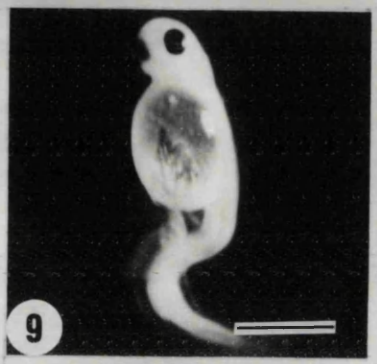
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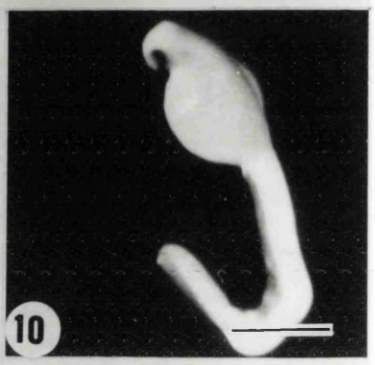
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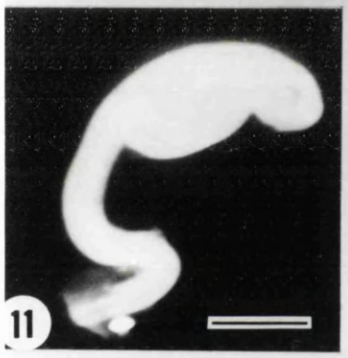
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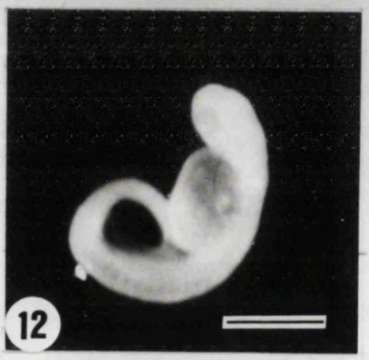
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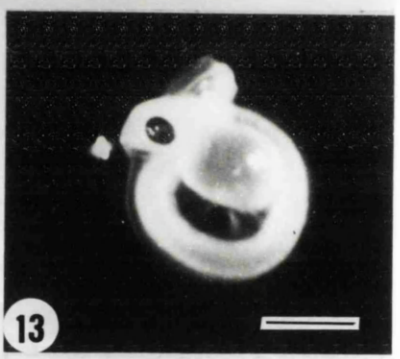
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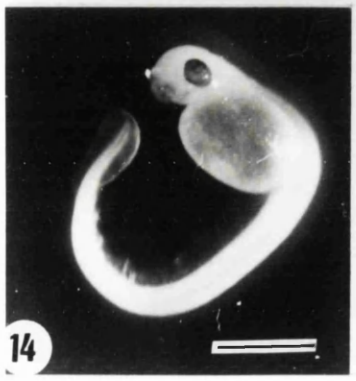
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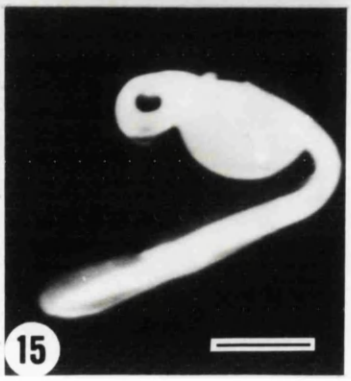
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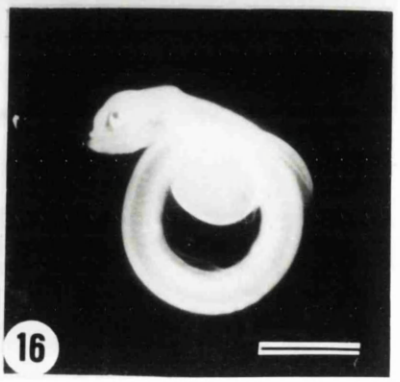

16

Photographs showing jaw abnormalities of the newly hatched larvae of *C. harengus*.

Figure 17. Photograph showing head of normal hatched larva.
Scale bar 0.2 mm.

Figure 18. Photograph showing head of larva with reduced upper jaw.
Scale bar 0.2 mm.

Figure 19. Photograph showing head of larvae with 'primary pugheadness'.
Scale bar 0.2 mm.

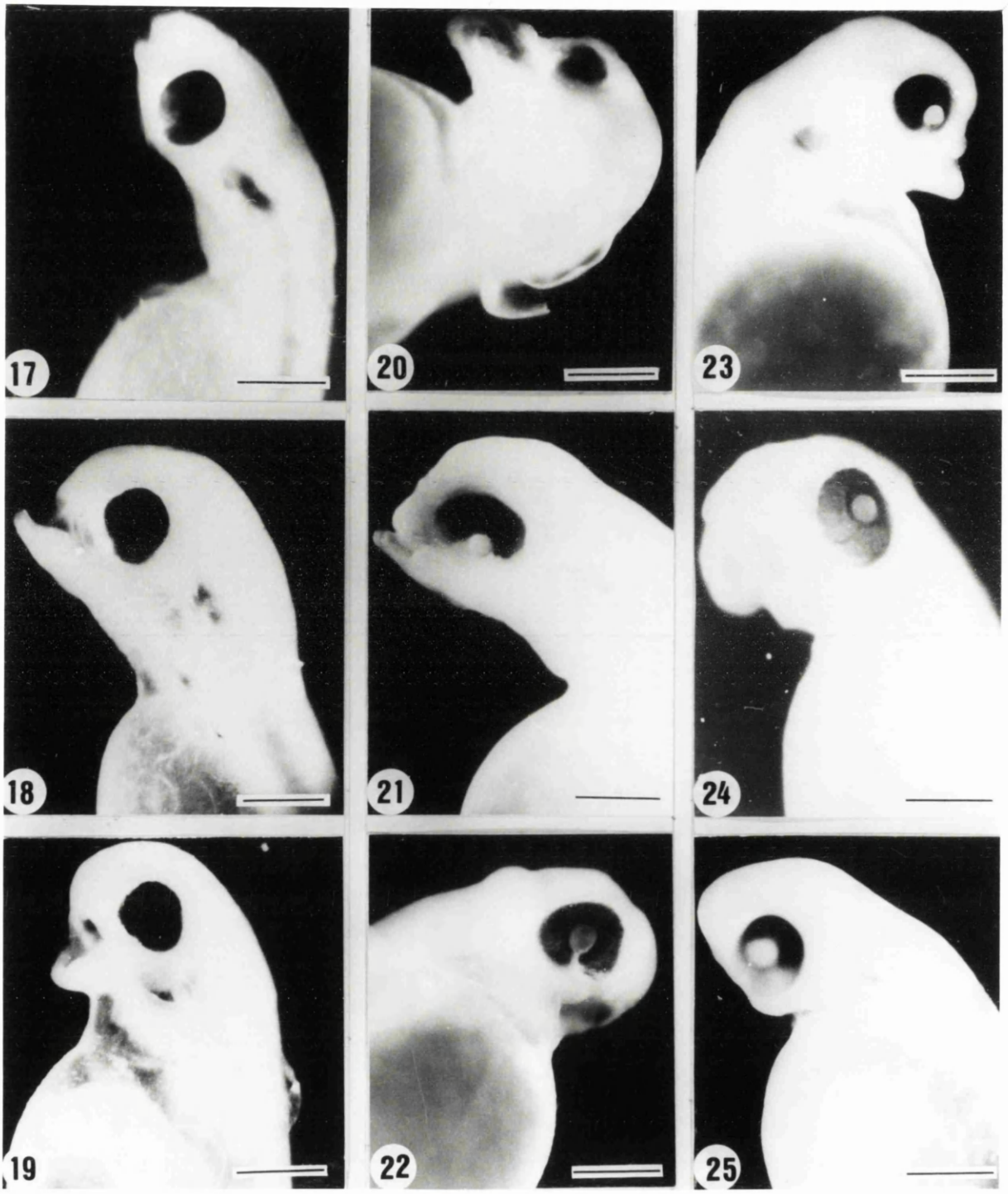
Figure 20. Photograph showing head of larva with enlarged upper and lower jaws.
Scale bar 0.2 mm.

Figure 21. Photograph showing head of the larva with reduced upper jaw.
Scale bar 0.2 mm.

Figure 22. Photograph showing head of larva with reduced upper and lower jaws 'sucker mouthed'.
Scale bar 0.2 mm.

Figure 23. Photograph of the head showing 'primary pugheadness'.
Scale bar 0.2 mm

Figure 24 & 25.
Photographs of the head showing 'exophthalmus'.
Scale bar 0.2 mm.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24
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22
25

Chapter 6

The effects of copper on the ultrastructure of brain cells, epidermal cells and muscle cells of *C. harengus* larvae

INTRODUCTION

Skin forms the external covering of a fish's body and is thus in contact with the outer environment. It consists of two layers, the epidermis and dermis. The epidermis consists of epithelial cells including a number of mucous cells, chloride cells and neuromasts (Jones *et al.*, 1966; Somasundaram, 1985; Mohammad-Nagib, 1987), whereas, the dermis consists of fibroelastic connective tissue with relatively few cells. The major function of the epidermal layers, especially the mucous cells, is to produce mucin, a glycoprotein substance which together with water, forms mucous, a thick, slimy, lubricating secretion. This secretion makes swimming easier for fish and protects them from parasites, bacteria and other small organisms (Lagler *et al.*, 1977). The chloride cells are columnar in shape, with a single nucleus, numerous mitochondria, an elaborate tubular endoplasmic reticulum and vesicles in the apical cytoplasm. These cells have roles in both ion secretion and absorption (Lubin *et al.*, 1989). It has been reported that respiration, osmoregulation and absorption occur through the skin in early larval stages, when gills are not yet functional, and in adult fish for a limited period (Shelbourne, 1957; Van Oosten, 1957; Holliday, 1964; Blaxter, 1969; Ferreira *et al.*, 1984). Blaxter and Holliday (1963) found that the central nervous system and epidermal tissues were functional at hatching in *Clupea harengus* L.

Beneath the skin there are two distinct layers of muscles, red and white. The red muscle forms a thin sheet immediately underneath the epidermis and above the white muscles (Somasundaram *et al.*, 1984).

The skin is the first and, initially at least, the most important target of heavy metal toxicity in fish larvae, especially when gills are

non-functional (Ormsaye and Brafield, 1984; Somasundaram, 1985; Mohammad-Nagib, 1987). Any disruption of the epidermis may affect osmoregulation and respiration or increase the chances of attack by micro-organisms. *C. harengus* L. has stocks which spawn in estuarine waters where heavy metal pollution may occur. Somasundaram *et al.* (1984a,b, 1985) have shown that eggs of *C. harengus* L. exposed to zinc produce larvae with deformities, an altered rate of development and cellular disruption in the epidermis, brain and trunk muscle tissue. Damage to brain tissue, dilation of the kidney tubules, renal necrosis around tubules, increased liver fat and enzyme inhibition and degeneration of the anterior lateral line and olfactory sensory structures were caused by copper toxicity (Peter, 1966; Gardner and La Roche, 1973; Gardner, 1975; Klink, 1975; Beckman and Zaugg, 1988). Gardner and La Roche (1973) observed cellular changes in *Fundulus hetroclitus* L. and *Menidia menidia* L. especially in the sense organs when adult fish were exposed to copper. Peter (1966) reported the destruction of brain cells in pigeons as a result of copper accumulation. Ultrastructural studies of gills and liver of fish have shown that damage is caused to the cells by copper (Baker, 1969; Segner, 1987). Ultrastructural changes in the epithelial cells, muscle cells and brain cells of *C. harengus* L. larvae exposed to both zinc and aluminium have been reported by Mohammad-Nagib (1987) and Somasundaram *et al.* (1984) and Somasundaram (1985). The present study was conducted to assess the possible effects of copper on the ultrastructure of the skin, muscles and brain cells of herring larvae, *C. harengus*.

Materials and Methods

C. harengus adults, which were about to spawn, were gill-netted from Castle Reach, Milford Haven, South Wales during March 1989. Eggs were stripped from these fish and artificially fertilized (Alderdice, 1979). Samples of approximately equal numbers of fertilized eggs were placed in 2 litre glass jars containing either clean artificial seawater (Tropical Marine Salts) diluted to $20^{o}/oo$ (ambient salinity) or test solutions containing copper in $20^{o}/oo$ artificial seawater. Solutions contained either 0.01, 0.03, 0.05 ppm copper, prepared from a stock solution of $CuSO_4.5H_2O$. The jars were continuously aerated at $8^{o}C$ (ambient temperature). Control and test solutions were renewed every 2 days to maintain the level of copper and to counter possible adsorption by glassware and uptake by the eggs. At hatching (15 days after fertilization), larvae were removed for electron microscopy fixation.

Electron Microscopy

Samples of ten larvae were removed from both control and test solutions and fixed for 2 h at $4^{o}C$ in 5% cacodylate buffered glutaraldehyde with sucrose added. These were then washed in several changes of the buffer solution at $4^{o}C$ for 24 h, post-fixed in chilled osmium tetroxide for 90 minutes, washed for a further 1 h in buffer solution, then dehydrated in a graded series of cold acetone and finally embedded in TAAB embedding resin.

Gold/silver interference colour sections were obtained using glass knives on a Cambridge Huxley mark I ultramicrotome and mounted on copper grids. The grids were double stained in 80% uranyl acetate in methanol (20 min) and lead citrate (10 min) and viewed in a Joel 1200ex transmission electron microscope. A morphometric analysis of the electron micrographs

was carried out according to Weibel *et al.* (1966). Electron micrographs were enlarged to a final magnification of 39 x 10^3 and analysed using a multipurpose test system consisting of 100 points enclosing 50 short test lines in order to determine the relative volume of the mitochondria and the surface to volume ratio of their cristae in chloride cells; the relative volume of muscle fibre, sarcoplasmic reticulum, mitochondria and the surface to volume ratio of cristae in muscle cells; the relative volumes of nucleus, mitochondria and perinuclear space in brain cells.

P (probability) was obtained by using student's t-test.

1. The effects of copper on the ultrastructure of brain cells of larvae of *C. harengus*.

Observations:

Brain cells of *C. harengus* larvae have a large nucleus, the cytoplasm is electron dense containing a large number of ribosomes, mitochondria, extensive endoplasmic reticulum and several golgi complexes (Fig. 1). The relative volumes of the nucleus, mitochondria and perinuclear space (the space between the outer and inner nuclear membrane) in control and copper exposed specimens is presented in Table (1).

The brain cells from specimens treated in 0.01 ppm copper showed no significant changes in the relative volumes of nucleus, mitochondria and perinuclear space compared with those from the control (Fig. 2). However, in specimens exposed to 0.03 and 0.05 ppm copper, the nuclei of the brain cells were relatively reduced in size ($P<0.001$) and the perinuclear spaces increased ($P<0.001$)(Figs. 3,4). Mitochondrial morphology was changed by swelling and sometimes breakdown of the cristae and the formation of vacuoles in the matrix. The mean mitochondrial size was reduced ($P<0.001$) in specimens exposed to both 0.03 and 0.05 ppm copper. The cytoplasm was

less electron dense in specimens from higher (0.05 ppm) concentrations of copper; there were fewer ribosomes and fewer golgi complexes (Fig. 5). The cisternae of the endoplasmic reticulum were dilated.

2. The effects of copper on the ultrastructure of the epidermal cells of larvae of *C. harengus*.

Observations

The epithelium of the larvae of *C. harengus* consists of three types of cell: (1)the outer epithelial cells; (2) inner epithelial cells; and, (3) chloride cells. Electron microscopic observations reveal that each epithelial cell of the outer layer contains an elongated nucleus, a small number of mitochondria, golgi complexes and vesicles. The distal surface of the outer epithelial cells is extended into microvilli or microridges (Figs. 6,7). Beneath these epithelial cells there is a felt-like mass of fibres forming a plate. The inner epithelial cells have extensive rough endoplasmic reticulum, however, other organelles are similar to those in the outer epithelial cells. The inner epithelial cells are connected to each other by desmosomes (Fig. 7).

Jones *et al.* (1966) failed to observe chloride cells in the epidermis of herring larvae, *C. harengus*, though Somasundaram (1985) observed them on the yolk sac, head and in the region of the trunk just above the yolk sac. Chloride cells contain a large number of densely packed mitochondria, each with numerous cristae and an electron dense matrix (Fig. 13). The cytoplasm contains an extensively branching tubular type smooth and rough endoplasmic reticulum which ramifies throughout the cell except for a narrow region in the apical cytoplasm. There are a number of free ribosomes in the cytoplasm. The nucleus, which is more or less spherical or ovoid in shape,

is located in a peripheral position in the cell. The free surface of the chloride cells is exposed to the environment, has a diameter of about 6.2 μm and lies in a depression on the skin surface, flanked by epidermal cells. Immediately below the external surface the cytoplasm contains a number of osmiophilic vesicles and tubules.

Significant morphological changes occurred in both epithelial and chloride cells in larvae hatched either from incubation in 0.03 or 0.05 ppm copper. The first change in the outer epithelial cells was the appearance of vacuoles in the mitochondria and the disappearance of the felt-like fibrous mass between the outer and inner epithelial cells (Fig. 8,9). In specimens hatched following incubation in a concentration of 0.03 ppm copper the endoplasmic reticulum and perinuclear spaces in the inner epithelial cells were dilated (Fig. 11). In specimens from solutions of 0.05 ppm copper the cytoplasm of the outer epithelial cells was vacuolated, the organelles disappeared except for some remnants of mitochondria and golgi complexes and there was disruption of the nuclear envelope. The epithelial layer became sloughed off and the microvilli were numerous compared with those in the controls (Figs. 10,12). The nuclei of the inner epithelial cells had low electron density. The cristae of mitochondria were fragmented or had disappeared.

The relative volume of the mitochondria and surface to volume ratio of their cristae in chloride cells of larvae treated in different copper concentration are shown in Table 2. The surface to volume ratio of the mitochondrial cristae was significantly greater in larvae hatched in 0.03 ppm copper than those of the controls. The smooth endoplasmic reticulum was swollen (Figs. 15). The chloride cells from larvae incubated in 0.05 ppm copper were smaller than those in the controls, contained fewer

mitochondria, formed nodular masses and contained granular and osmiophilic inclusions (Fig. 16). The mitochondrial cristae degenerated leaving a granular mass in the matrix (Fig. 14).

3. The effects of copper on ultrastructure of trunk muscle cells of larvae of *C. harengus*.

Observations

The general organisation of the muscle layers in herring larvae, *C. harengus*, is summarised by Somasundaram *et al.* (1984). The red muscle layer lies immediately beneath the epidermis, followed by a white muscle layer. The sarcoplasmic reticulum in both muscle layers forms a network of inter-connected cisternae around the myofibrils. The sarcoplasm contains a large nucleus, mitochondria and ribosomes. The mitochondria contain closely packed cristae and an electron dense matrix (Figs. 17,18). The mean relative volumes of the myofibrils, sarcoplasmic reticulum, mitochondria and the surface to volume ratio of cristae in control and copper exposed specimens is presented in Table 3.

Morphometric analysis showed no significant difference in the muscle cells of larvae hatched following incubation in 0.01 ppm copper and those of the controls, however, individual mitochondria and sarcoplasmic reticulum were swollen. The major changes were observed in specimens from 0.03 and 0.05 ppm copper. In specimens hatched in 0.03 ppm copper, the sarcoplasmic reticulum showed a significant swelling (<0.001) and the perinuclear space was increased compared with those of the controls. The mean relative volume of the mitochondria showed a significant increase in size, and contained degenerated cristae (Fig. 19,20). The surface to volume ratio of the mitochondrial cristae was reduced ($P<0.05$). In specimens hatched from 0.05

ppm copper, there was a significant reduction in the surface to volume ratio of myofibrils ($P<0.001$) compared with specimens from controls (Fig. 21,22). The relative volume of mitochondria and the surface to volume ratio of cristae were both significantly reduced ($P<0.001$) and the sarcoplasmic reticulum was swollen ($P<0.001$).

DISCUSSION

Many of the chemical wastes, including heavy metals which are discharged into the aquatic environment may exert neurotoxic effects on the sensory organs of teleosts. These neurotoxic effects are significant whether they cause permanent neurological damage or temporary disability (Gadner, 1975). In the present study on herring larvae the brain cells are affected by copper. The morphology of their organelles is altered. Somasundaram *et al.* (1984) and Mohammad-Nagib (1987) described similar cellular damage in the mid and hind brain of *C. harengus* larvae when they were exposed to either zinc or aluminium. Copper was toxic to the gill epithelial cells of *Pseudopleuronectus americanus* Walbaum. The cytoplasm of the cells was vacuolated by the formation of vesicles and lysosomes and a dense granular material accumulated in the cells which was sometimes deposited within the mitochondria (Baker, 1969). Ultrastructural changes of hepatocytes in the roach, *Rutilus rutilus* L., were also observed when the fish was exposed to copper. In these cells, the cisternae of the endoplasmic reticulum and perinuclear space were dilated, mitochondria were swollen and were less electron dense and the number of their cristae reduced (Segner, 1987). Copper inhibits the activity of liver enzymes in *F. heteroclitus* L. (Jackim *et al.*, 1970). Pathological effects of copper were recorded in *F. heteroclitus* L. The chemoreceptor sites of olfactory organs

were damaged, vasodilation and congestion occurred in all parts of the brain with haemorrhage and rupture of the meninx (Gardner and La Roche, 1973). Many fish species have shown physical stress in copper solutions by changes in their normal behaviour associated with locomotion, feeding, avoidance and coughing (Drummond *et al.*, 1973; Kleerekoper *et al.*, 1973; Scarfe, 1982; Steel, 1983; and Hartwell *et al.*, 1989). Peter (1966) reported the toxic effect of copper on the brain of pigeons. He suggested that the biochemical changes in mitochondria and changes in the potential of the cell membrane of brain cells during copper exposure inhibit ATP synthesis and ion exchanges through the membrane. Trump *et al.* (1975) described mercury binding to SH groups of the cell membrane, resulting in an increase in the permeability of the membrane to cations and water allowing entry into the cell which causes swelling of the mitochondria, endoplasmic reticulum and nuclear envelope. Heavy metals affect the activity of membrane ATP-ase enzyme of the cells which may cause changes in the structural organisation of cellular organelles. These changes in mitochondrial membranes and a reduction in ribosomes may lead to an inhibition of protein synthesis (Bubel, 1976; Somasundaram *et al.*, 1984). Na^+, K^+, ATP-ase enzymes are generally thought to be major components in the biochemical mechanism for ion transport in all animal tissues. Copper inhibits Ca^+, Na^+ and K^+ uptake and reduces the whole body ion concentration and disrupts the normal osmoregulatory function (Lauren and McDonald, 1987b; Sayer *et al.*, 1989; Reader *et al.*, 1989) and it also inhibits Na^+, K^+-ATPase enzyme (Buckman and Zaugg, 1988). In *Leponis maxochirus* Rafinesque, copper caused an increase in water content in muscle and liver which resulted in a decrease in ATP concentration of the tissues due to dilution of cellular metabolites (Heath, 1984). Exposure of the roach, *R. rutilus* to copper caused alteration in mitochondrial morphology

which may represent a very early demarcation of future pathological development (Segner, 1987). In the present study changes in the morphology of the organelles of brain cells may disrupt their normal function causing a reduction in the supply of ATP to the cell. Copper may also affect brain functions by inhibiting many enzymes which are necessary for brain development.

The presence of microridges and microvilli on the skin have been reported in many teleost larvae including *C. harengus* as also found in the present study (Jones *et al.*, 1966; Roberts *et al.*, 1973; Matty *et al.*, 1980; Lubin *et al.*, 1989; Foscarini, 1989). Bereiter-Hahn *et al.* (1979) concluded that the formation of microridges involves a folding of the plasmalemma brought about by a force generated by filaments inserted at the prospective flank regions of the developing microridges. Forscarini (1989) described the formation of the microridges in oviparous fishes, these start from the peripheral area of the epithelial cells and progress towards the centre. Blaxter (1969) and Forscarini (1989) found that the microridges or microvilli contribute to respiratory and excretory activities before the gills become functional and physiologically they are sensitive to stimuli in the detection of the surrounding environment. In the present study epithelial cells of larvae treated with 0.05 ppm copper had numerous microvilli which were comparatively longer than those in the controls (Fig. 12). However, on the surface of the degenerated outer epidermal cells, the microvilli have disappeared (Fig. 10). Somasundaram (1985) reported a clumping together of microridges near the periphery of epidermal cells to form a high ridge in larvae of herring, *C. harengus*, exposed to 6.0 ppm zinc solutions. The high microridges were also observed when larvae of *C. harengus* were exposed to mercury (Jastania, 1989). Olson *et al.* (1973)

reported that mercuric chloride or methyl mercuric chloride can cause structural damage to gill epithelia by a loss of surface microvilli.

In the present study necrosis and sloughing off the epidermal cells were observed in 0.05 ppm copper solutions. The outer epidermal cells were vacuolated. The mitochondrial cristae were reduced and the mitochondria reduced in size. Jones *et al.* (1966) observed no change in the ultrastructure of the skin of *C. harengus*, subjected to different salinities. In *C. harengus* larvae, hatched from eggs previously incubated in either 6.0 or 12.0 ppm zinc, the epidermal cells contained more vesicles, intracellular spaces and their mitochondria were swollen significantly compared with those in the controls and showed signs of necrosis (Somasundaram, 1985). Nuclear pycnosis, necrosis and sloughing off of epidermal cells of larvae of *C. harengus* were also reported in larvae treated in acidic and aluminium solutions (Mohammad-Nagib, 1987). Baker (1969) reported that in the winter flounder, *P. americanus*, when exposed to copper there was a cytological breakdown of the gill epithelium which began to slough off expanding chloride cells into bubbles. Numerous vesicles and empty vacuoles and granular material occurred within the mitochondria of gill epithelial cells. Eisler and Gardner (1973) reported necrosis of the epithelial lining of the oral cavity of *F. heteroclitus* exposed to zinc. Wobeser (1975) found that rainbow trout, *Salmo gairdneri* Richardson when exposed to mercury, showed severe epithelial necrosis, swelling of mitochondria and nuclei and hyperplasia. Bilinski and Jones (1973) and Beckman and Zaugg (1988) suggested that any disruption of cellular structure in the gill epithelium is the primary cause of the inhibition of ATP-ase enzymes or the enzymes of the tricarboxylic acid cycle in mitochondria.

In the present study the chloride cells observed were similar to those reported in *F. heteroclitus* (Kessel and Beams, 1960, 1962). Chloride cells contain two principle cytoplasmic inclusions, numerous mitochondria and an interdigitating system of rough and smooth endoplasmic reticulum. Lasker and Threadgold (1968) suggested that these cells may be concerned with osmoregulation because of their ultrastructure, position within the larval skin, time of appearance and changes in microtubular structure and cell volume under salinity stress. In the present study chloride cells were affected by copper, the relative volume of mitochondria and surface to volume ratios of cristae were reduced in 0.05 ppm copper concentrations. Mohammad-Nagib (1987) reported a cellular degeneration of the chloride cells in the epidermis of larvae of *C. harengus* caused by acidic solutions. Baker (1969) recorded chloride cells in the gills instead of mucous cells when *P. americanus* were exposed to copper. He suggested that the replacement of chloride cells in copper exposed specimens may be related to the adaptation and excretion of copper. Oronsaye and Brafield (1984) reported that an increase in the number of chloride cells may be a response to the increase in general ionic regulation at the gill following damage to the kidney by cadmium, but the decline in their number suggests that they or the cells from which they arise, were being poisoned by cadmium and their effectiveness in removing cadmium reduced with long-term exposure. Somasundaram (1985) suggested that the appearance of chloride cells is related to an environmental adaptation and their disruption may be due to changes in metabolic activity. He found an increase in mitochondrial cristae at low (0.5 ppm) concentrations of zinc while in high (12.0 ppm) concentrations there was a reduction in the size of the mitochondria and endoplasmic reticulum.

In the present study a disruption of ultrastructure and the sloughing off of the epidermal cells in high concentrations of copper (0.03 or 0.05 ppm) may affect respiration and osmoregulation, thus reducing the viability of the larvae since these cells are functionally involved in osmoregulation and respiration (Blaxter, 1969; Ferreira *et al.*, 1984).

In the present study copper induced ultrastructural changes in the muscle cells. The mitochondria of the cells were swollen in specimens from low (0.03 ppm) concentrations but their size was reduced in specimens from higher (0.05 ppm) concentrations. Similar changes in muscle cells were observed when larvae of herring, *C. harengus* were exposed to zinc or aluminium (Somasundaram *et al.*, 1984; Mohammad-Nagib, 1987). Cameron and Smith (1980) described an increase in the number of mitochondria and swelling of individual mitochondria in the trunk muscles of the larvae of *C. pallasi*, exposed to crude oil. The swelling of mitochondria may be due to osmotic disorder of the cell (Rouiller, 1960). Copper interferes with mitochondrial functions which results in changes in mitochondrial morphology (Zaba and Harris, 1978; Segner, 1987). Trump *et al.* (1975) suggested that the swelling of membrane bound organelles is the result of an accumulation of cations, anions and water in the cell. De Robertis *et al.* (1970) suggested that swelling of mitochondria results from inhibition of oxidation and changes of osmotic pressure caused by inorganic phosphate. A swelling of mitochondria and endoplasmic reticulum was also reported in Isopoda, *Jaera nordmanni* (Rathke) by Bubel (1976), caused by the effects of heavy metals on the membrane ATP-ase system.

In the present study sarcoplasmic reticulum swelled and there was a reduction in the volume of myofilaments and muscle fibres of muscle cells. Bubel (1976) reported a reduction in the number of ribosomal particles by

the effect of heavy metals which may affect protein production. Somasundaram *et al.* (1984) suggested that the reduction in protein synthesis may be the cause of the reduction in myofibrils. At 2 ppm concentrations of copper elicited changes in oxygen-dependent ATP production by acting directly on biochemical reactions in the muscle tissue (Heath, 1984). The exposure of fish to copper caused a depression of the oxidation process by inhibiting enzymatic activity which resulted in a reduction of skeletal muscle metabolism and disruption of cellular organisation (Hubschman, 1967; Bilinski and Jones, 1973; Smith, 1982). Heath (1984) and Jackim *et al.* (1970) reported an increase in water content of muscle tissue and impaired cellular respiration when fish are exposed to copper, which may lead to metabolic impairments and can be a cause of cellular degeneration. Reduced growth and weight are common results of copper effects on fish (Mount *et al.*, 1969; McKim and Benoit, 1971; Takeshimurai *et al.*, 1981; Collvin, 1985). Ellgaard and Guillot (1988) reported that the metabolic rate of juveniles of the blue gill, *L. maxochirus* Rafinesque, was affected at copper concentrations as low as 0.01 ppm and induced locomotor hypoactivity.

Copper in concentrations of 0.03 to 0.05 ppm affected the components of muscle cells in the larvae of *C. harengus*. In the present study a change in mitochondrial morphology, reduction in myofibrils and disruption of sarcoplasmic reticulum occurred, all of which would impair movement and hence affect the viability of the larvae.

TABLE 1. MORPHOMETRIC ANALYSIS OF THE RELATIVE VOLUMES OF NUCLEUS, MITOCHONDRIA AND PERINUCLEAR SPACES IN THE BRAIN CELLS OF *CLUPEA HARENGUS* LARVAE

Concentrations (ppm) (Cu)	N	Mean Relative Volumes		
		Nucleus	Mitochondria	Perinuclear Space
Control	10	0.393	0.060	0.040
.01	10	0.400 (>0.1)	0.055 (>0.1)	0.038 (>0.1)
.03	10	0.250 (<0.001)	0.035 (<0.001)	0.893 (<0.001)
0.05	10	0.210 (<0.001)	0.032 (<0.001)	0.110 (<0.001)

N = No. of observations

Significant difference from the control where $P < 0.05$.

TABLE 2. MORPHOMETRIC ANALYSIS OF RELATIVE VOLUME OF THE MITOCHONDRIA AND SURFACE TO VOLUME RATIO OF THEIR CRISTAE IN THE 'CHLORIDE CELLS' OF *C. HARENGUS* LARVAE.

Concentrations ppm (Cu)	N	Mean relative volume of mitochondria	P	Mean surface to volume ratio of cristae	P
Control	15	0.60		0.42	
0.01	15	0.61	>0.1	0.46	<0.05
0.03	15	0.53	<0.05	0.33	<0.05
0.05	15	0.46	<0.01	0.20	<0.001

N = No. of observations

P = Significance of differences in relation to control.

TABLE 3. MORPHOMETRIC ANALYSIS OF RELATIVE VOLUMES OF THE MUSCLE FIBRES, SARCOPLASM RETICULUM, MITOCHONDRIA AND THE SURFACE TO VOLUME RATIO OF THE MITOCHONDRIAL CRISTAE.

Concentrations ppm (Cu)	Muscle fibre N		P	Mean relative volume of sarcoplasm	P	Mitochondria	P	Surface to volume ratio of cristae	P
Control	15	0.46		0.046		0.24		0.60	
0.01	15	0.47	>0.1	0.048	>0.1	0.23	>0.1	0.57	>0.1
0.03	15	0.40	<0.05	0.052	<0.001	0.29	<0.001	0.49	<0.05
0.05	15	0.21	<0.001	0.130	<0.001	0.18	<0.01	0.33	<0.001

N = No. of observations.

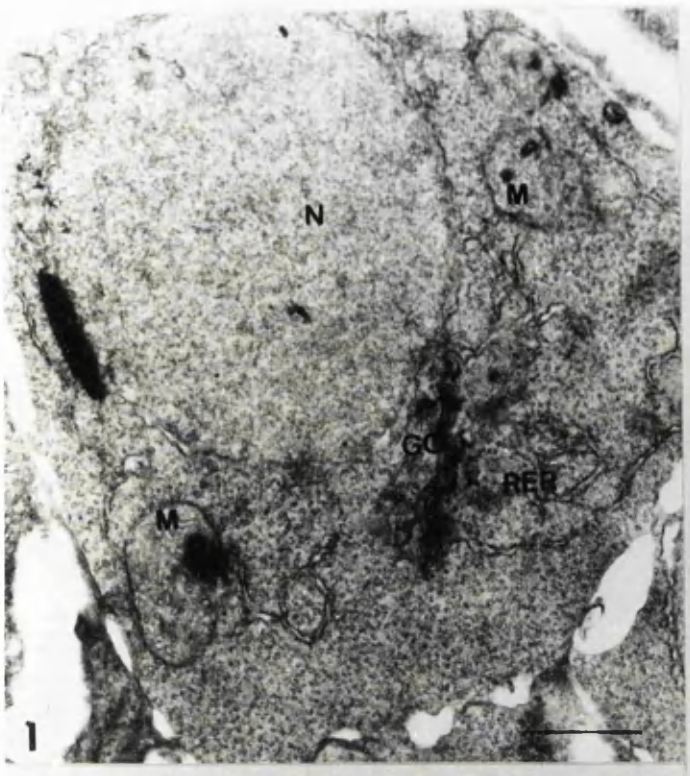
P = Significance of difference in relation to control.

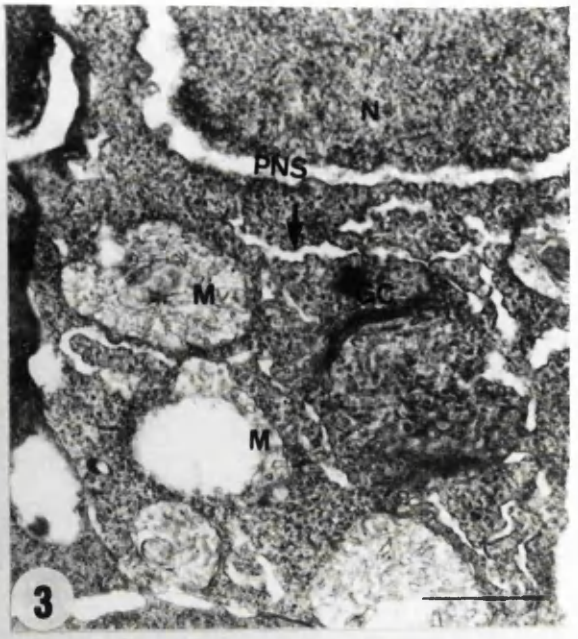
Figure 1. Electron micrograph of brain cells of *C. harengus* larvae at hatching showing a large nucleus (N), mitochondria (M), golgi complexes (GC), rough endoplasmic reticulum (RER) and electron dense cytoplasm with free ribosomes.
Scale bar 1 μm.

Figure 2. Electron micrograph of brain cells of *C. harengus* larvae hatched from eggs previously exposed to 0.01 ppm copper, showing large nucleus (N), mitochondria (M) and rough endoplasmic reticulum (arrow).
Scale bar 1 μm.

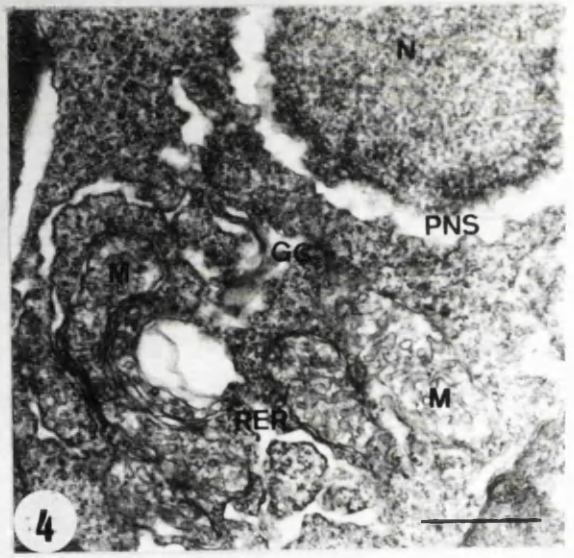
Figure 3. Electron micrograph of brain cells of *C. harengus* larvae hatched from eggs previously exposed to 0.03 ppm copper, showing golgi complex (GC), swollen mitochondria (M), swollen rough endoplasmic reticulum (arrow) and swollen perinuclear space (PNS). Note the mitochondria contain large vacuoles and low electron-dense matrix.
Scale bar 1 μm.

Figure 4 & 5. Electron micrograph of brain cells of *C. harengus* larvae hatched from eggs previously exposed to 0.05 ppm copper, showing a small electron-dense nucleus (N), an enlarged perinuclear space (PNS) and cytoplasm containing swollen rough endoplasmic reticulum (RER), degenerating golgi complexes (GC) and swollen mitochondria with degenerating cristae.
Scale bar .5, 1 μm.


N
M
GC
RER
M
1


N
PNS
M
GC
M
3


M
N
2


N
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M
RER
4

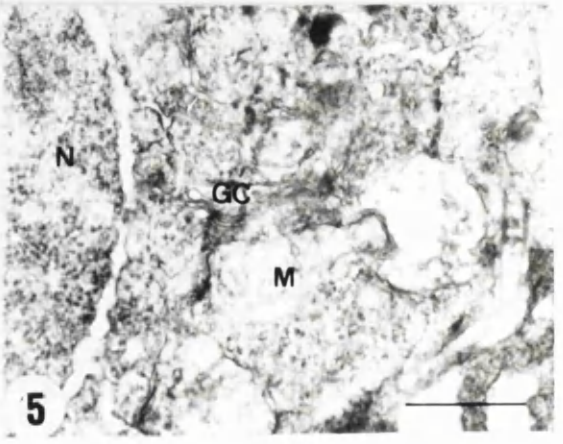

N
GC
M
5

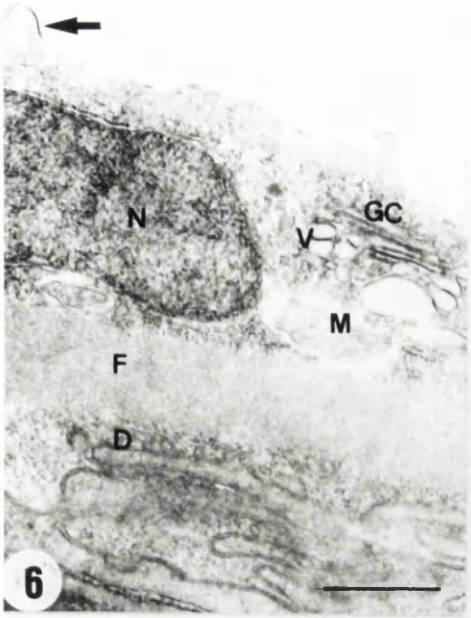
Figure 6. Electron micrograph of the epidermal cells of the skin of *C. harengus* larvae at hatching, showing the outer epidermal cells. Each cell contains nucleus (N), mitochondria (M), golgi complexes (GC), vesicles (V) and felt-like mass of fibres (F) forming a plate. Arrow head = microvilli; D = desmosomes.
Scale bar 1 μm.

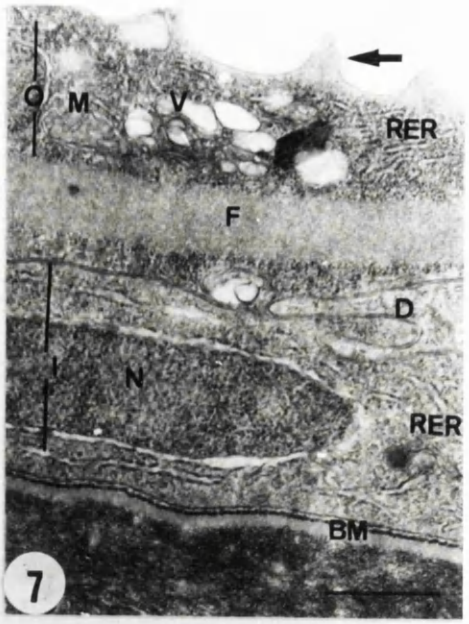
Figure 7. Electron micrograph of epidermal cells of *C. harengus* larvae at hatching, showing the outer (O) and inner (I) epidermal cells. Outer epidermal cells contain rough endoplasmic reticulum (RER) and nucleus (N). The epidermal cells are connected together by desmosomes (D). BM = basement membrane.
Scale bar 1 μm.

Figure 8. Electron micrograph of epidermal cells of *C. harengus* larvae hatched from eggs previously exposed to 0.03 ppm copper, showing the vacuoles (VC), degeneration of mitochondria (M) and disappearance of felt-mass. D = desmosomes.
Scale bar .5 μm.

Figure 9. Electron micrograph of epidermal cells of *C. harengus* larvae hatched from eggs previously exposed to 0.05 ppm copper, showing sloughing of outer epidermal cells. The cytoplasm is dilated and contains vacuoles (VC), distorted mitochondria and numerous microvilli (arrow). The felt-like mass has disappeared.
Scale bar 2 μm.

Figure 10. Electron micrograph of the epidermal cells of *C. harengus* larvae hatched from eggs previously exposed to 0.05 ppm copper, showing degeneration of outer epidermal cells. The cytoplasm contains remnants of mitochondria (small arrow) and golgi complexes (GC). The nuclear membrane is disrupted (N). Large arrow = microvilli. Scale bar 2 μm.


N
GC
V
M
F
D
6


O
M
V
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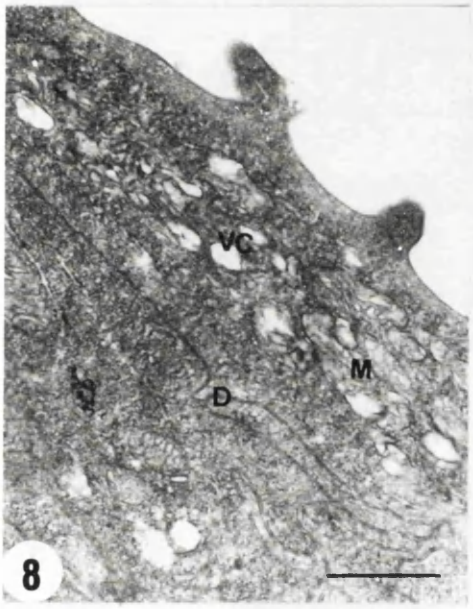
D
I
N
RER
BM
7


VC
M
D
8


M
VC
9

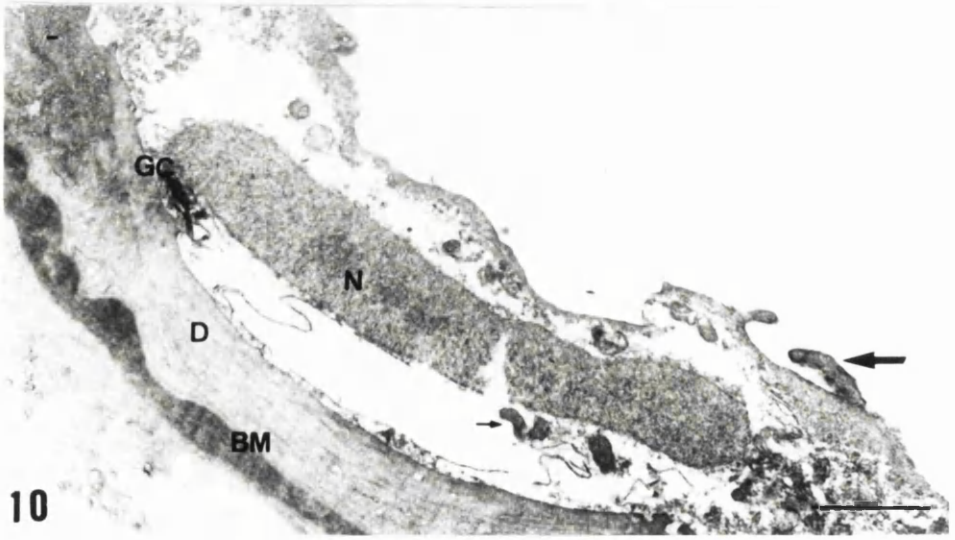

GC
N
D
BM
10

Figure 11. Electron micrograph of epidermal cells of a *C. harengus* larvae hatc from eggs previously exposed to 0.03 ppm copper and showing fragmentation of rough endoplasmic reticulum (RER), mitochondria (M and the perinuclear space (◄—) of inner epidermal cells.
Scale bar 1 μm.

Figure 12. Electron micrograph of epidermal cells of a *C. harengus* larvae hatc from eggs previously exposed to 0.05 ppm copper, and showing increasing abundance and size of microvilli (MV).
Scale bar 500 nm.

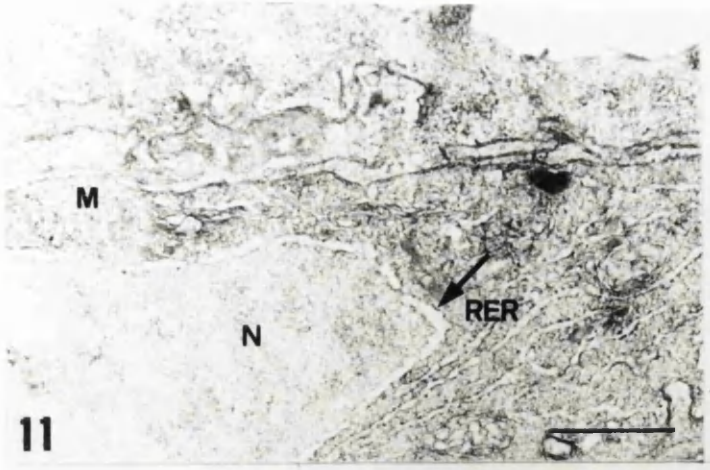
Figure 13. Electron micrograph of chloride cells in the epidermis of a *C. harengus* larvae at hatching. The epidermis contains extensively branched smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (big arrow) and numerous mitochondria (M). Note the chloride cell extending beneath the epidermal cells (E) and connect to the surface (small arrow) via the epical pit (AP). V = vesicles BM = basement membrane.
Scale bar 1 μm.

Figure 14. Electron micrograph of chloride cells of a *C. harengus* larvae hatch from eggs previously exposed to 0.05 ppm copper, and showing the degeneration of mitochondrial cristae (M). RER = rough endoplasmic reticulum.
Scale bar 1 μm.

Figure 15. Electron micrograph of chloride cells of a *C. harengus* larvae hatch from eggs previously exposed to 0.03 ppm copper, and showing swolle smooth endoplasmic reticulum (SER) and mitochondria (M).
Scale bar 1 μm.

Figure 16. Electron micrograph of chloride cells of *C. harengus* larvae hatched from eggs previously exposed to 0.05 ppm copper and showing the reduced size and abundance of mitochondria (M) and granular inclusions (GI) in the cytoplasm. SER = smooth endoplasmic reticulum.

Scale bar 1 μm.



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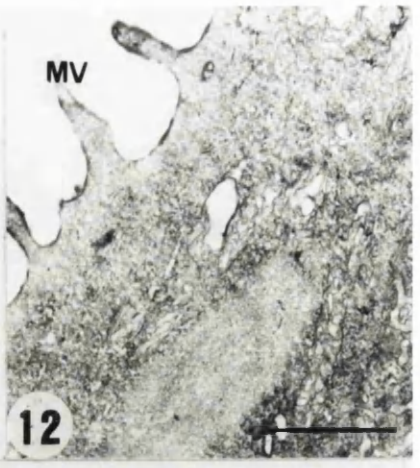

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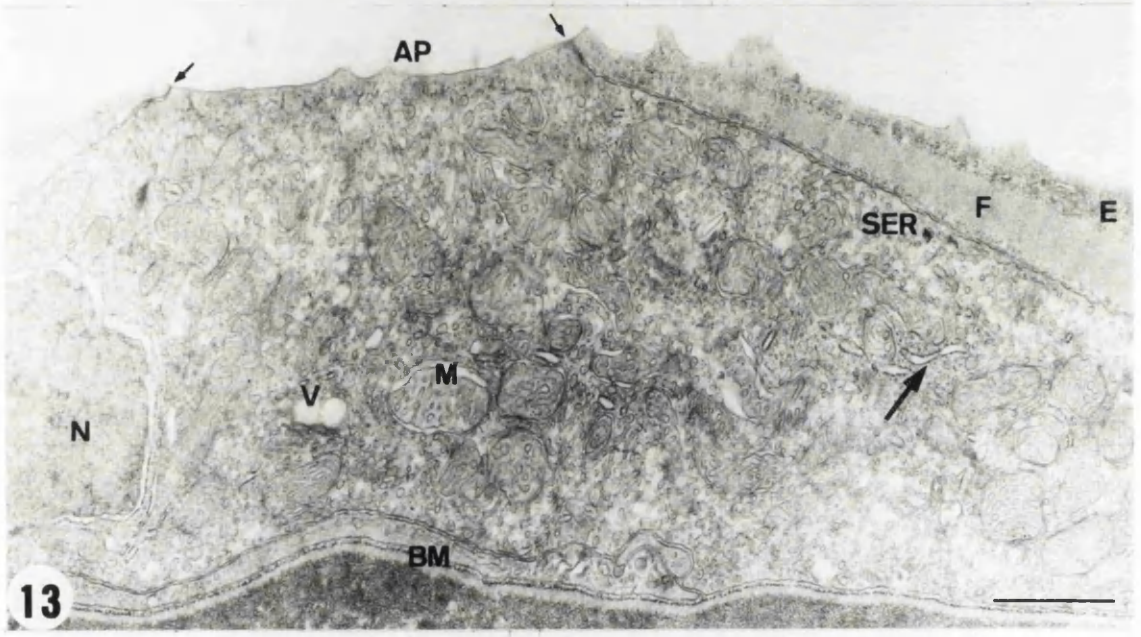

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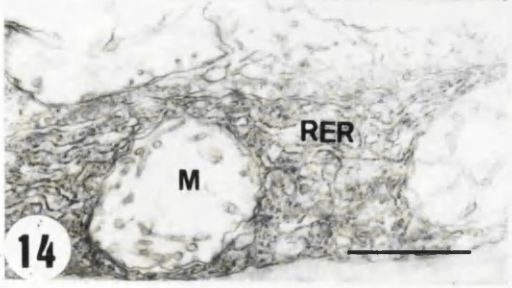

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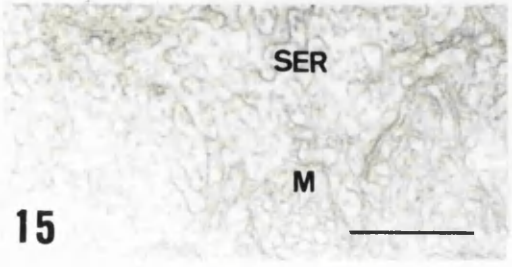

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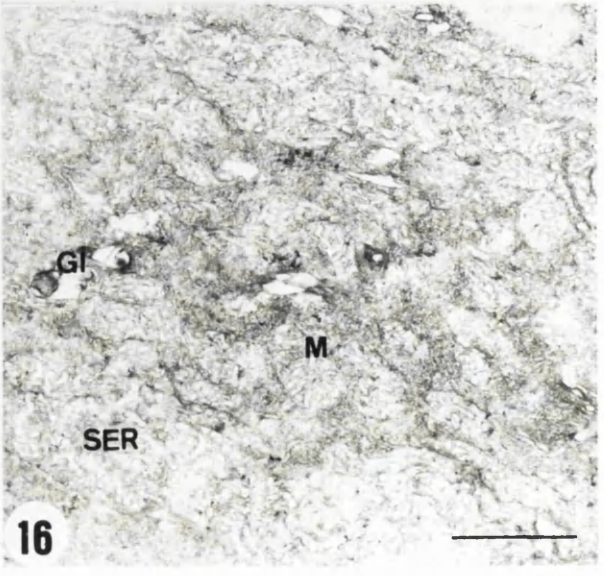

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Figure 17. Electron micrograph of trunk muscle tissue of a *C. harengus* larvae at hatching showing red muscle fibres (FI) beneath the epidermis (E). The muscle cells contain mitochondria (M), nuclei (N) and sarcoplasmic reticulum (arrow).
Scale bar 1 μm.

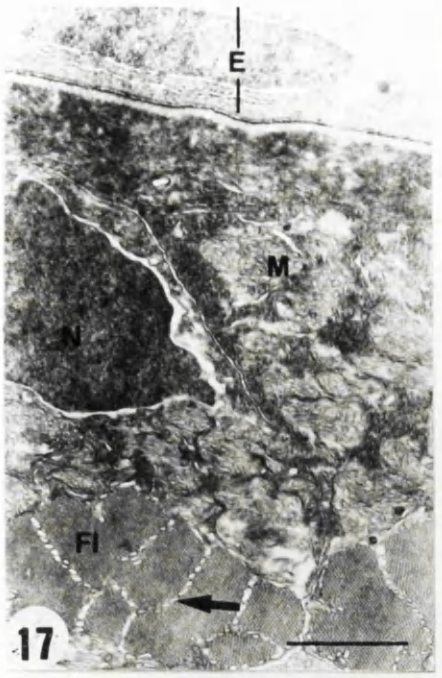
Figure 18. Electron micrograph of trunk muscle of *C. harengus* at hatching showing a white muscle layer which contains a large fibre (FI). Note the sarcoplasmic reticulum (arrow), nuclei (N) and mitochondria (M).
Scale bar 1 μm.

Figure 19. Electron micrograph of trunk muscle of a *C. harengus* larvae hatched from eggs previously exposed to 0.03 ppm copper, showing swollen sarcoplasmic reticulum (SR). FI = myofibrils.
Scale bar .5 μm.

Figure 20. Electron micrograph of red muscle of a *C. harengus* larvae hatched from eggs previously exposed to 0.03 ppm copper, showing swollen mitochondrial cristae (M) and a perinuclear space (PNS). FI = myofibrils; N = nucleus.
Scale bar .5 μm.

Figure 21. Electron micrograph of red muscle of a *C. harengus* larvae hatched from eggs previously exposed to 0.05 ppm copper, showing swollen sarcoplasmic reticulum (arrow) and swollen mitochondria (M) with degenerating cristae. FI = myofibrils. Scale bar .5 μm.

Figure 22. Electron micrograph of trunk muscle of *C. harengus* larvae hatched from eggs previously exposed to 0.05 ppm copper, showing swollen sarcoplasmic reticulum (SR) and degeneration of mitochondrial cristae (M). FI = myofilaments. Scale bar .5 μm.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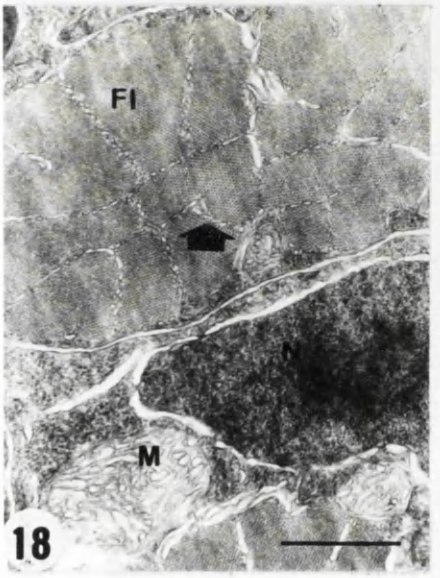
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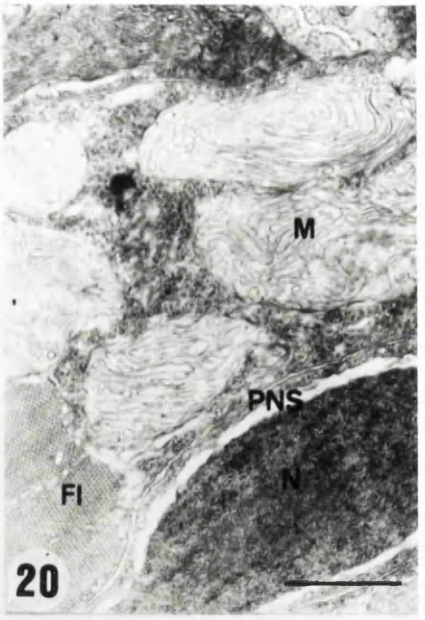
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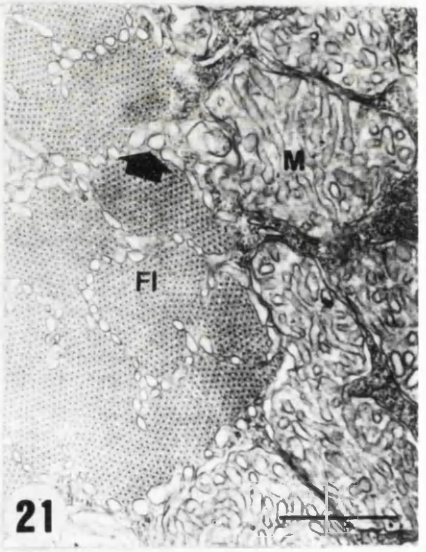
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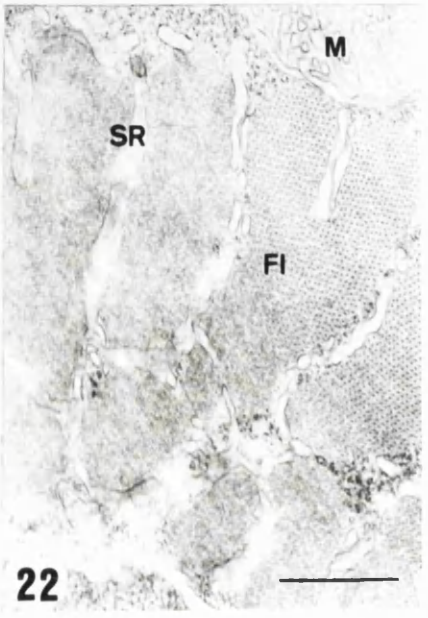
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Chapter 7

General Discussion

GENERAL DISCUSSION

Coastal waters and estuaries are highly productive habitats which provide feeding or spawning grounds for numerous fish species. Fishes occurring in these habitats are categorised as:

(i) Estuarine species which spawn in the sea.

(ii) Marine species with seasonal migrations into the estuary as adults.

(iii) Marine species using the estuaries as a nursery area.

(iv) Diadromous species which spend most of their lives in fresh water and migrate to estuaries at some stage of the life cycle.

Fish species live in an environment which exhibits a multitude of physical, chemical and biological constraints. Thus those species are continually exposed to many environmental factors such as temperature or salinity acting independently or in concert with pollutants (Elliott *et al.*, 1988). Each species exists in an environmental compromise and is adapted to survive within certain ranges of environmental factors (Sheehan, 1984). Environmental conditions may not always be in the optimal range to the organisms for all essential body functions. The following aspects can be indicative of any environmental threat to fish *viz.* ecology, biochemistry, bioaccumulation studies, pathobiology, genetics, behaviour and physiology of fish (McIntyre and Pearse, 1980).

Since most commercial fish species do not spend their whole life in estuaries and coastal waters, it was necessary to study more than one species at the same time recognizing that juveniles and adults of all species do not necessarily react to environmental changes in the same way. Thus the present study investigated food, population structure and reproductive biology of grey mullet species feeding in coastal and estuarine

waters, and the effects of heavy metals on eggs and larvae of herring which spawn there.

Grey mullets, thick-lipped, *Chelon labrosus* (Risso) and thin-lipped, *Liza ramada* (Risso) are heavily dependent on estuaries and coastal waters which provide suitable feeding grounds collectively for the juveniles and adults. Studies of food and feeding of mugilids have been reported from different parts of the world (Bishop and Miglarese, 1978; Chan and Chua, 1979; Kraul, 1983; Romer and McLachlan, 1986; Wijeyaratne and Costa, 1987). Mullet feed at the lowest trophic levels on diatoms and other microalgae (Odum, 1970), and obtain their energy directly from the first trophic level (Oren, 1981). The difference in diet of grey mullets are quite evident according to age and size (Kraul, 1983; Romer and McLachlan, 1986; Wijeyaratne and Costa, 1987). Food of grey mullet, *Mugil tade* (Forskäl) was considerably different when studied in different environments, fresh water tanks, brackish water, estuaries and the sea (Pillay, 1953). Juveniles of the majority of grey mullet are initially carnivorous feeding on copepods and other zooplankton until they reach a length of about 3 cm S.L. (Nash and Shehadeh, 1980; Kraul, 1983), however, early juveniles of *M. tade* up to 2 cm S.L. feed mainly on algae (Myxophyceae)(Pillay, 1953). Suzzuki (1965) reported the variation in feeding behaviour with increasing length of *M. cephalus* L. which is initially a zooplankton feeder, then a mixed food feeder (plant and animal) and finally a plant feeder.

The present study of stomach contents of *C. labrosus* at various stages of growth indicates that a transition in food occurs as the fish grows. Juveniles of size 3 to 5.1 cm S.L. feed mostly on copepods and some diatoms. The juveniles 5.2 to 8 cm S.L. feed on a mixed diet consisting of copepods,

diatoms and other microalgae, and detritus. Juveniles over 8 cm S.L. take similar food to that of adults consisting of benthic copepods and other benthic crustacea, microalgae and detritus mixed with sand and silt. The nature of their food indicates benthic feeding is occurring. The present study shows that crustacea are the most important food item for the juveniles and adults of *C. labrosus*. This was also reported by Hickling (1970), Casserre *et al.* (1975), Blaber (1976) and Bishop and Miglarese, 1978).

Spawning of *C. labrosus* and *L. ramada* takes place in offshore localities. Juveniles of *L. ramada*, size 4-4.9 cm S.L. reach the coastal waters of the Bristol Channel in early August, becoming 5.9 cm S.L. by October. Juveniles of *C. labrosus* are 6.1 cm S.L. in late autumn in the same sites (Claridge and Potter, 1985). Kennedy and Fitzmaurice (1969) caught juvenile *C. labrosus* from Irish waters as early as 15th July with a range of 1.5-2.0 cm S.L. In October they were in the size range 2.5-6.5 cm S.L. According to Hickling (1970), juveniles of *C. labrosus* grow from about 2 cm in August to 4-4.5 cm S.L. by December in Plymouth estuaries. In the present study the smallest juveniles of *C. labrosus* collected in December had a mean length of 4 cm S.L. The difference in the size of juveniles of *C. labrosus* from different localities in British waters may be due to differences in spawning period between the areas. On the basis of the times when young *C. labrosus* were collected from Irish waters and Plymouth estuaries, spawning could occur in British waters between the end of May and the end of September (Kennedy and Fitzmaurice, 1969; Demir, 1971). In the present study the gonadosomatic index indicates that *C. labrosus* spawns between April to May and *L. ramada* December to January. Hickling (1970) reported autumn as the spawning period for *L. ramada* in the English Channel.

Demir (1971) discussed evidence regarding the spawning period of *C. labrosus* and concluded that it depends on the conditions of the water such as temperature, which vary from year to year or from region to region.

The present study indicates that *C. labrosus* is a slow growing species. The biggest fish caught was 57 cm total length and sixteen years old. Growth of fish depends mostly on environmental conditions such as temperature and food availability and quality. Most marine fishes grow little in winter in temperate waters and some virtually cease growing during the colder months. The growth of the common grey mullet, *M. cephalus* in the Bosphorus and the Sea of Marmara was rapid from May to September but slow after the end of October (Erman, 1959). Growth of the same species ceased during mid-winter in Australian waters (Thomson, 1951). Kennedy and Fitzmaurice (1969) suggested that the cessation of growth in *C. labrosus* was caused not only by a reduced rate of metabolism caused by low temperature but also by a partial winter fast. They did not find any growth between specimens caught in spring and those in the previous autumn of the same age groups. In the present study the condition factor gradually increased during summer, reached a peak in August, and then declined during the winter months to reach a minimum in February. This indicates that the growing conditions are more favourable for the fish in summer than in winter.

In the present study the smallest mature males and females of *C. labrosus* were 37 and 40 cm total length respectively. The males matured at about 8 and the females at about 10 years of age. Difference in size at first maturity in the grey mullet species has been reported in warm and cold waters. The grey mullet in warmer waters became mature at an earlier age and smaller size than those in the colder waters (Kennedy and Fitzmaurice, 1969; Thong, 1969b; Hickling, 1970; El Maghraby *et al.*, 1974b). The size at

first maturity is probably influenced by the local mean sea temperature. For example, pond-cultivated grey mullet, *M. cephalus*, receiving heated effluents from a power station had fully developed gonads at one year old, length 16 cm S.L. (Linder *et al.*, 1975).

Gonad development can be influenced by fluctuations in the external environment such as salinity, temperature, oxygen or by pollutants (Hoar and Randall, 1969; Köhler, 1981; Somasundaram *et al.*, 1984). Reproductive failure, retarded sexual development and spawning were prevented in *Salvelinus fontinals* (Mitchill) and *Pimephales promelus* (Rafinesque) when exposed to hard fresh water containing copper (Mount, 1968; McKim and Benoit, 1971). Since these effects were observed on reproduction of fishes it was important to look at the oogenesis in a fish living in coastal waters and estuaries, which would be a basis for further study on pollutant effects.

The present study examined the oocyte development in *C. labrosus* and seven stages were identified at the ultrastructural level. Oocyte development has also been described in a number of other marine teleosts in the Bristol Channel, for example, *Blennius pholis* L. (Shackley and King, 1977), *Dicentrarchus labrax* L. (Mayer, 1987), *Gaidropsarus mediterranus* L. and *Ciliata mustela* L. (Mohammad-Nagib, 1987). In these species eleven, eight, seven and six stages respectively were identified by ultrastructural study. The stages are characterised by the ultrastructural changes in the oocyte structure throughout development. These stages can differ between winter and summer spawners or between pelagic and benthic species. Oocyte development can also differ between species which have one or more spawning

periods in a year, or which spawn only once in a lifetime (semelparous species).

Ultrastructural differences were also found to occur between species in the structure of the developing chorion, the zona radiata. In *B. pholis* and *D. labrax* the zona radiata is tripartite (Shackley and King, 1977; Mayer *et al.*, 1988), whilst in others, *G. mediterraneus* and *C. mustela* it is monopartite and bipartite (Mohammad-Nagib, 1987). These structural variations may be correlated with differences in salinity, temperature and viscosity of the seawater necessitating adjustments in the structure of pelagic eggs to secure adequate buoyancy (Lönning, 1972) or may be correlated with the degree of abrasion experienced by the egg when spawned in the littoral or offshore environment (Mohammad-Nagib, 1987).

In the present study the zona radiata of *C. labrosus* consists of a distinct tripartite structure. The first formed zona radiata externa is of two homogeneous, electron-dense layers, whilst the later formed zona radiata interna is a broad and highly-organised fibriller structure. The difference in the densities of zona radiata externa and interna is due to the difference in proportion of carbohydrates and proteins contained in each zone (Anderson, 1967).

In the present study the lipid yolk droplets appear in the oocyte before the protein yolk granules. Lipid yolk is believed to be endogenous in origin, formed by the endoplasmic reticulum and golgi complexes. The first appearance of lipid yolk has also been reported in other marine teleosts, *B. pholis* and *D. labrax* (Shackley and King, 1977; Mayer et al., 1988). However, the appearance of protein yolk before lipid yolk was confirmed by Mohammad-Nagib (1987) in *G. mediterraneus* and *C. mustela*.

Cortical alveoli form at the same time as lipid yolk and are also endogenous in origin, formed by the endoplasmic reticulum and golgi complexes. In the later stages of vitellogenic oocytes (550-700 μm) cortical alveoli were observed to fuse with the oolemma and release their contents (cortical reaction). This premature discharge could be due to artificial activation by the fixative (Shackley and King, 1977).

Protein yolk formation occurred after that of lipid yolk formation. Ultrastructural evidence suggests that the sources of protein yolk are a combination of both exogenous and endogenous contributions. Exogenous protein precursors are accumulated by pinocytosis through the oolemma, whilst the endogenous protein contribution is synthesised by golgi complexes and endoplasmic reticulum in a similar way to that described in other teleosts, *viz. Lebistes reticulatus, Salmo gairdneri* and *Chromis chromis* (Droller and Roth, 1966; Beams and Kessel, 1973; Donato *et al.*, 1980).

Estuaries and coastal waters can be vulnerable to any contaminant materials including heavy metals. In addition, there is usually considerable fluctuation in environmental factors such as temperature and salinity. Heavy metals mostly occur in sewage and industrial effluents, which are major routes of pollutants to coastal waters. At suitable low concentrations some heavy metals are essential for enzymatic activity. They also form a group of enzyme inhibitors when trace concentrations are exceeded. Non-essential metals such as Ag, Hg, Cd, Pb, and essential metals such as Cu, Fe and Zn are particularly toxic and usually inhibit enzymes by forming mercaptides with the sulphydryl group which are responsible for catalytic activity (Vallee and Wacker, 1970). Consequently, most heavy metals, whether essential or not, are potentially toxic to living organisms. Coastal and estuarine organisms tend to accumulate heavy metals from the

environment through direct contact or food (Bryan, 1971). Recently reviewed reports of skeletal deformities in fish populations from California, New York, Japan and West Germany indicate that the anomalies are related to heavy metal or chlorinated hydrocarbon pollution (Sindermann *et al.*, 1980). In a number of studies copper, an essential heavy metal, caused pathological conditions in adult fish and mortality of eggs and larvae (McKim and Benoit, 1971; Gardner and La Roche, 1973; Blaxter, 1977). In previous studies eggs and larvae of *Clupea harengus* L. were affected by zinc, aluminium and mercury (Somasundaram, 1984a,b, 1985; Mohammad-Nagib, 1987; Jastania, 1989). The present study demonstrates that copper affects the viability and hatchability of *C. harengus* eggs which cause failure of recruitment and will reduce the populations. Fertilized eggs of *C. harengus* show a significant decrease in the total egg volume and that of the perivitelline space at 0.03 and 0.05 ppm copper. This reduction of egg volume may be due to a reduction in osmoregulation which results in a decrease in the uptake of water (Peterson and Martin-Robichand, 1984). Premature hatching of *C. harengus* eggs occurred by two days in 0.03 and 0.05 ppm copper solutions. This premature hatching produced small and deformed larvae. Abnormalities which appeared in these larvae were vertebral flexure, otic and eye malformations and jaw and branchial deformities. Similar effects have also been observed by Westernhagen *et al.* (1979) and Somasundaram *et al* (1984b) in the development of *C. harengus* eggs when exposed to cadmium, copper, lead and zinc solutions. All these malformations would affect the survival potential since the larvae would be unable either to maintain equilibrium during swimming due to skeletal curvature or to perform the prey capture manoeuvres necessary for feeding and growth (Rosenthal and Alderdice, 1976), as they possess only rudimentary upper and lower jaws. These effects would

thus reduce the ability of the larvae to exploit the environment and consequently reduce the overall viability of the larval population.

The present study also describes copper-induced ultrastructural changes in epidermal cells, muscle cells and brain cells of *C. harengus* larvae hatched from eggs previously exposed to 0.03 and 0.05 ppm copper. The larvae showed epidermal necrosis and degeneration of chloride cells which would reduce cutaneous respiration, osmoregulation and metabolism. Chloride cells have been identified as the site of active chloride secretion and high ionic permeability (Foskett and Scheffey, 1982). This is indicated by the presence of an extensively branched tubular system of smooth endoplasmic reticulum and abundant mitochondria containing closely packed cristae which are essential for ion transport against a concentration gradient.

Muscle cells showed swollen and disrupted sarcoplasmic reticulum which would affect muscle contractability. This was supported by reduced muscle fibres accompanied by degeneration of mitochondrial cristae which indicate a lower metabolic activity (Bubel, 1976) this would impair movement and affect the viability of the larvae. Copper in muscle tissue also caused loss of calcium from cell membranes, altering membrane permeability which leads to an increase in water content of the intercellular fluid (Heath, 1984; Jackim *et al.*, 1970).

Brain cells showed degeneration of organelles such as mitochondria, endoplasmic reticulum, golgi complex and degeneration of the nucleus which would cause disability of brain tissue to perform normal functions. Disablement of sensory perceptions would make the larvae more susceptible to enemies and predation, disease or other hazards due to an inability to relate to, or cope with, a viable environment.

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